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# SOCIAL BEHAVIOR AND THE EVOLUTION OF MAN'S MENTAL FACULTIES

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## INTRODUCTION

The concept of the uniqueness of man is again in the forefront of biological thought. The basis of that uniqueness is recognized as man's capacity for culture, its accumulation and transmission. This capacity in turn is the resultant of the unique mental qualities man displays, qualities which set him off from even his nearest relatives among the primates. This point of view has been brilliantly elaborated by several modern students of evolution, particularly by Julian Huxley (1941) and by G. G. Simpson (1949). Henry Nissen (1951) has phrased it (p. 426) in behavioral terms.

> With one notable exception the phylogenetic course of behavioral development has been gradual; that it has been a continuous affair, proceeding by quantitative rather than qualitative changes. The one exception is that which marks the transition from the highest nonhuman primates to man (that is, "modern man"). At this point a new "dimension" or mode of development emerges, culture or "social heredity."

If this uniqueness of man is to be understood in terms of evolutionary biology it can only be as the resultant of a biological history that includes unique conditions under which the basic driving forces of evolution have operated in his history. Since modern biology has returned to considering natural selection as the basic driving force in evolution we must expect to find the explanation of man's separateness in some features of his history by which a selective pressure was accorded to the particular forms of intelligence and cooperative behavior which mark man as a culture-capable organism. Furthermore these selective pressures must have come into play upon an organism which provided the kind of genetic variability which permitted evolution to progress in the requisite direction. Any adequate theory of human evolution must specify both of these factors.

The general discussions of the post-Darwinian era sought an explanation of man's culture-accumulating ability in pointing out the general survival value of intelligence, particularly in such activities as tool using. The recent emphasis on the biological significance of cooperative behavior by Allee (1951), Montagu (1950) and others stresses the value to the group of

such intra-group cooperation. These generalized discussions while of course valid do not offer an explanation of the special characteristics of man's intelligence or social cooperation since they do not qualify on either of the two counts mentioned above. Indeed in their more enthusiastic presentations these points of view lead one to wonder why all animals are not as intelligent as Einstein and as moral as Schweitzer.

Whereas biologists are mostly content to treat the problem of the evolution of human mentality from that of a primate ancestor in general terms, anthropologists usually skip over it entirely and assume the culture-accumulating capacity of man as the basis from which their discussions start. For example, Tappen (1953) writing recently on a mechanistic theory of human evolution states:

> Ancestors of the human group must have made the shift over to symbolic communication to initiate specifically human evolution. Such an adaptive change corresponds to Simpson's evolutionary mode, the *quantum evolution*. Once such a shift toward this new adaptive zone was initiated, a high selective advantage for individuals better adapted to learned behavior and symbolic communication must have ensued.

It is evident that from the biological point of view the crux of the matter is the explanation of the shift over to symbolic communication which is assumed as given by Tappen.

Dobzhansky and Montagu (1947) have briefly considered this problem and have offered the suggestion that human intelligence and cooperative behavior have developed in connection with the adjustment of individuals to each other within the social group. They emphasize that this dependence of human mentality upon social behavior implies that the same essential factor has operated on the evolution of mentality in all human groups in contradistinction to the variety of environmental factors which have operated to produce physical diversity in different races. Their fundamental contribution is thus to account for the general conformity of all races in mental characteristics in spite of a degree of physical distinctiveness. Their point of view is also important in that it orients the search for the selective factors in the evolution of man's mentality toward the analysis of social behavior. But their brief consideration does not attempt to specify the behaviors involved in the selective process.

Chance and Mead (1953) in a recent study have attempted to specify the social behavioral factors involved in man's evolution. They point out the role of the cortex in control of autonomic function. They consider that the young primate male, subject to competition for place in an autocratic primate society such as that of the baboon, has a high survival value placed upon the cortical control of his relations, particularly the autonomic control of emotional behavior. Hence they suppose a considerable selection pressure is exerted for the expansion of the cortex. Apparently their concept is that human mentality, otherwise than as it is characterized by cortical dominance of autonomic function, is an incidental product of the expansion of the cortex. They say (p. 437):

**We therefore conclude that the ascent of man has been due in part to a competition for social position, giving access to the trigonal sphere of social activity in which success was rewarded by a breeding premium, and that at some time in the past, a group of primates, by virtue of their pre-eminent adaptation to this element and consequent cortical enlargement became pre-adapted for the full exploitation of the properties of the mammalian cortex.**

This theory seems of limited applicability for a number of reasons. It is based on one aspect of social behavior, intense male competition for dominance in a polygamous group. This, though characteristic of baboon and macaque society, is not universal among primates. It hardly seems applicable to precultural man for reasons to be discussed later. Perhaps a more fundamental defect of the theory is that it is non-adaptational, making the various aspects of human intelligence incidental to selection in respect to other characteristics which, it should be pointed out, operate in the male sex only. Such a view is hardly consistent with modern emphasis on selection pressure as the basic motive power of evolution (Dobzhansky, 1937; Mayr, 1942).

## THE BASIS FOR A SELECTION PRESSURE THEORY

Any attempt to develop a theory to account for the distinctive traits of intelligence and cooperative behavior as shown by man is necessarily highly speculative. Yet the importance of this subject for an understanding of the biology of man is so great as to justify renewed attempts whenever advances in related fields give new insights that may be helpful. The recent advances in the paleontology of man, particularly in respect to the South African man-apes, the Australopithecines, and Pekin man, Sinanthropus, have given new and unexpected light on the characteristics of man at the period of the origin of his culture-developing capacities. The recent burgeoning of interest in the study of the comparative psychology and the social behavior of vertebrates, especially of the primates, has afforded new concepts that enable us to deal with the problem in concrete terms. It will be our purpose here to draw upon these fields for materials from which to formulate a theory of some of the selective factors operating on man in the transition from the non-cultural to the cultural level.

It is not necessary for us to accept any fixed notions as to the exact position of the australopithecines in respect to man's evolution. Whether in direct line of evolution of modern man or not, we can accept them as representing a stage or level of evolution in which the anthropoid-to-man line showed the following characteristics. The animal had a brain not much larger than that of our present great apes, with a cranial capacity of perhaps 600–800 cc., a body somewhat smaller than modern man's (about 100 lbs.), canines not conspicuously enlarged nor dimorphic, erect posture and bipedal locomotion leaving the hands free of locomotor use. The abundance of baboon and other mammalian bones and evidence of cracking of long bones and the skulls in a way to permit extraction of their contents as food strongly suggest that these African man-apes hunted baboons and smaller mammals as food.

On the basis of these aspects of his australopithecine finds, Dart (1949) inferred that these man-apes were hunters who lived in part by killing fairly large and ferocious mammals (the baboons of the kitchen-middens were somewhat larger than present-day types). He reasoned that the man-ape could not have effectively hunted them unless he used tools. Since no clear stone artifacts are associated with their remains, it must be presumed that such tools were of wood or bone or consisted of naturally occurring stones. Bartholomew and Birdsell (1953) have considered the social organization of australopithecines in connection with population problems and have accepted Dart's point of view. They point out the significance of tool use in exploitation of food resources.

The use of tools was greatly emphasized by Darwin (1871) and other early writers as the basis of the natural selection of intelligence in human evolution. Carveth Read (1920) suggested that precultural man was a wolf-ape, running in wolf-like packs to hunt various moderate and large mammals. He too emphasized the success that would attend those individuals better able to make and use tools in the hunt. But he also stressed the selective value of cooperative behavior in this ecological niche, particularly the development of leadership and discipline in the ranks.

As we have seen, the fossil evidence of australopithecines gives definite support to these older speculations. We can therefore accept as part of the basis for a modern hypothesis of the social organization of precultural man the following points. He was a bipedal terrestrial hunting organism before he developed the human brain. The free use of his hands in tool use and tool production gave a selective advantage to increased intelligence. Possibly he practiced pack hunting and thus was exposed to selection in terms of group cooperative behavior. Such considerations help us to see some of the selective actions that led to the so-called "explosive" evolution of the human brain (Eiseley, 1953, Weidenreich, 1946).

When we turn to consider the Sinanthropus level of human evolution we again may point out that we do not necessarily regard this fossil as in direct line to a modern race but as representative of a particular level of human ancestry. The significant characteristics of that level are a brain of approximately 900–1000 cc., associated with early paleolithic artifacts, and clear evidence of the use of fire in cooking. As Weidenreich remarked of Sinanthropus (1947), his eating habits cannot have differed greatly from those of primitive tribes today. The cultural artifacts associated with Sinanthropus appear to be equivalent to an early paleolithic (Mousterian) level. Davidson Black (1934) remarks on the small improvement seen over the whole extent of the deposits in contrast to the marked improvement shown in Spanish cave deposits covering a much shorter period. This possibly indicates that Sinanthropus brain was functionally, as it was volumetrically, not fully equal to that of modern man.

Middle pleistocene man, either Paleoanthropic (Neanderthaloid) or Neanthropic (Swanscombe, Fontechevade) displays a brain as large as that of the largest of modern races (Montagu, 1951). It may be that the Neanderthal

brain did not show as great a development of frontal and temporal-parietal association areas as that of contemporary man, but certainly the Neanthropic types showed brains which in their gross morphology are completely modern. Culturally too, there appears to be no justification for imputing any inferiority as compared to present-day paleolithic cultural man. Presumably then these fossil men were as capable of absorbing any level of culture, including that of the atomic age, as we presume contemporary primitives to be.

As biologists then we can regard the evolutionary span from australopithecines (presumably late pliocene or earliest pleistocene) to middle pleistocene man as the locale of the evolutionary change in brain and mentality which marks the transition from non-cultural anthropoid society to cultural human society. We can further accept from our knowledge of australopithecines the concept that before his mind made the transition man's ancestors had become erect, ground, perhaps pack running, hunters, dependent upon tools and cooperative pack behavior to supplement the stratagems characteristic of mammalian predators.

Turning now to recent developments in comparative psychology, I would accept two main points from the studies of Köhler, Yerkes and the many others who have explored primate behavior. The first is in regard to their intelligence. The higher apes, the chimpanzee in particular, are capable of insightful solutions of problems. However, these insight experiments and the results of delayed response and token-using experiments indicate that imagery or ideational behavior are part of the ape's mental equipment. However, the ape is very limited in this respect in comparison to man (Köhler, 1927; Yerkes, 1943). Chimpanzees show considerable individual variation but at best seem to be poorly able to maintain clear images of past events or to form images of future possibilities. Their insightful solutions of problems are good only when they are formed by combining elements already present. This low development of ideation is a principal factor in the failure of apes to develop true speech. One of the characteristics of speech at its lowest level is the representation by a symbol of concrete items not sensually present at the moment. The failure of apes to develop language in the human sense is today recognized as being based on no physical or physiological limitation but rather on psychological inadequacy. This is demonstrated most dramatically in the failure of baby chimpanzees raised in human families to make much progress in developing true speech beyond the use of two or three verbal symbols (Kellogg and Kellogg, 1933; Hayes, 1951). Even these symbols seem to be used chiefly when the objects referred to are present. Of course, the limitations of the ape mentality as compared to human depends on many other factors than the ability to form clear images of past or future events but for our present purpose we shall find this lack, which necessarily limits speech development, the most significant for our own thinking.

A second area in which we will find the concepts of comparative psychology of value is that relating to the socialization of the higher apes. In this connection we wish to point out that though dominance behavior is

clearly evident in ape social relations it is subject to modification in a number of respects of which female estrus deserves specific consideration here. Under natural conditions the male of a pair would be expected to dominate the female since, though the species is quite variable, the males generally are larger than females (Nissen, 1931). Under experimental conditions it has been found that the dominant male is modified in his behavior toward a female when she is in estrus (Yerkes, 1939). Then the male yields place to the female thereby permitting her to receive food rewards from which he regularly excludes her when she is not in estrus. Experimentally this relation has been shown to be hormonally controlled (Birch and Clark, 1946) and to apply to female-female pairs as well as to the male-female pairing (Crawford, 1940). Yerkes termed this phenomenon privilege granting, but in view of the demonstration of the complexity of the phenomenon (Birch and Clark, 1950) a more neutral term such as dominance modification is preferable. It is evident that ecologically dominance modification in estrus fits in with the common phenomenon of a closer social tie between male and female at the time of female estrus. It is a common phenomenon in primates that dominance relations between mates are modified in a way which permits closer socialization of male and female at this period (Carpenter, 1942; Zuckerman, 1932).

From the recent work on social behavior of animals, the primary insight I would select for our purpose here is the realization in concrete terms of the essential role played by social behavior in the adaptation of an animal to its environment (Tinbergen, 1951). The sexual, parental and other group behaviors are seen to be adjustments to the conditions of the animal's life. They are as necessary to its survival as any of its physiological or structural characteristics and therefore as much subject to natural selection. The classic study of the red deer by Fraser Darling (1936) is an example of the ecological significance of behavior.

A second fundamental proposition is that the elements of the social behavior of an animal form an integrated whole in which each element fits to the others and to the structure of the animal (Scott, 1944; Tinbergen, 1953). Consequently one can make inferences from one aspect, behavioral or structural, to another with the same sort of assurance (or lack thereof) as we are accustomed to do in going from one structure to the other. Illustrations of these propositions are best given as we develop the details of a concrete theory of protohominid social organization and the evidence for it.

## THE INTEGRATED FAMILY THEORY OF PROTOHOMINID SOCIOLOGY

The theory that is here suggested is that the protohominid at the australopithecine level of development lived in integrated family units, essentially monogamous, in which the male and female performed separate economic functions but were closely integrated by behavioral mechanisms into a unit in respect to parental functions. The male is visualized as being specialized as a hunter as well as a food gatherer and the female as being a food

gatherer and domestic. They shared a more or less permanent domicile. In the following paragraphs the basis for this inference is derived from considerations of the principles of social behavior particularly as seen in other mammals. The selection pressures operating in such a social organization are then analyzed from the point of view of the extent to which they can be seen to be pushing the man-apes in evolution in the direction of truly human mentality.

The male mammal is commonly poorly integrated into such social activities as the species possesses (Alverdes, 1927). In some solitary species as cats and rodents the male and female associate only during the female's estrus when insemination takes place. The female alone cares for the young. However, there are many types of mammalian societies, particularly in group-living forms, in which the male plays a more conspicuous social role. In the red deer at the rutting season the males leave their own groups and seek out the females. Each one herds as many females as possible into a small harem which he defends from all other males. At the close of the mating season however he breaks contact with the females and returns to the male haunts. The females form well-integrated herds in which the young are raised.

Such a social organization is characterized by males highly dominant over females and extremely aggressive toward other males during the mating season. Such males are obviously subjected to a selection pressure putting a high premium on fighting behavior, strength, aggressive temperament and weapons. This, of course, correlates with the structure of these creatures, sexual dimorphism being especially marked with respect to the male's equipment for fighting and for display.

In primate groups where the males remain with the group of females permanently a modification of this pattern obtains. In some species as the baboons and macaques the dominance and aggressive behavior of the males is as highly developed as in the deer tribe. Such males are larger than females, aggressive in temperament and with conspicuously developed canines. In such cases the males function in the group chiefly as defenders of the group and its territory against outside aggression. They play little or no positive role in domestic affairs, the raising of the young being entirely the burden of the females. In fact the aggressions of the males against each other and against females are a constant source of disturbance within the group, so much so, in fact, as to render impractical the maintenance of the group under semi-natural conditions (Carpenter, 1942; Zuckerman, 1932). Howling monkeys and chimpanzees show considerably less male aggressiveness and a rather greater contribution of the males to group welfare (Carpenter, 1934; Nissen, 1931). Correspondingly these species show less extreme sexual dimorphism.

It is evident that the reason the domestic burden can be effectively carried by the individual females without the help of the male in these primates is because these species live as food-gatherers, eating primarily plant and small animal materials. The young, born usually one at a time,

are small enough for the female to carry with her without serious interference with her foraging activities. The young in turn are equipped with grasping limbs enabling them to cling effectively to the belly of the mother with relatively slight danger of dislodgement. When the young attain a size at which they become a burden to the mother they attain independent locomotion.

Such behavioral organization whereby the female carries practically the entire burden of raising the young is possible in carnivorous animals as well but usually requires some special modification of behavior. In the cats such as the cougar, the female continues her hunting after littering but the indications are that she confines her foraging to the immediate neighborhood (Seton, 1929). At any rate the period of infancy is short and the female evidently survives it on limited rations. Later when the young can travel she covers her kill and leads the young to it. In the fur seal, fish-hunting on the part of the nursing female is permitted by a remarkable adaptation. The young can gorge themselves on milk at one feeding to survive several days while the female is away on a fishing expedition in nearby waters (Scheffer and Kenyon, 1952).

Wolves and other canines on the other hand attain a fundamentally different behavioral adjustment (Young and Goldman, 1944). In these species the male is integrated into the family group as a cooperating member that assists the female and young. Male and female pair up in the fall, apparently while still members of a pack. Later they separate from the pack, mate and clear out a number of dens. After birth of the young the male not only stands guard for the family but hunts for them as well. He may gorge himself at a kill and then disgorge at the den mouth for the female and young to feed on. Later the pair may hunt together, lead the young to the prey and gradually build them up to a family pack.

It is obvious that in so far as the protohominid was a hunter, the wolf type of behavioral adjustment is more suited to him than is that of the cat or seal. The anthropoid baby is too large to be carried on a hunt and requires too long a developmental period to permit a temporary tiding over of the nursing period. The mature anthropoid female is characterized by the fact that she is almost continuously carrying a child. As one is weaned the next is born. The female therefore cannot be an effective hunter. The development of a hunting economy can occur in an anthropoid only if the male cooperates in feeding and care of the young. The presupposition of monogamy for pre-cultural man which is here made is based on the impracticality of a single male operating successfully as protector and provider for many females. In animal societies in which the male maintains a harem as in the red deer, fur seal or baboon he devotes his continuous attention to guarding his harem against approach of other males. Such continuous guarding is of course impossible in the hunting economy visualized here for precultural man. Such considerations however, do not apply once the cultural level with its distinctive behavioral controls is reached.

The central feature of the social behavior of the "hunter" anthropoid therefore, must be an integration of the male into the monogamous family unit in which he is the primary hunter. This may be described as the wolf-type of behavioral adjustment, not however in the sense of Carveth Read, who apparently thought only of pack behavior and not of the domestic life of the wolf. There is, however, as we saw before, no reason to exclude the idea of pack hunting. It may well be that the males of several family units or of the extended single family covering several generations constituted a clan and hunted together. In that case the reasoning of Read (1920), Keith (1949), Bartholomew and Birdsell (1953) and others regarding the selective factors operating might be applicable.

## THE SELECTION PRESSURES OPERATING IN THE INTEGRATED FAMILY UNIT

In this section I will try to show that the biology of the integrated family unit as described above places a premium on the types of intelligence and cooperative activities that characterize human in contradistinction to ape behaviors. Such a selection pressure operating on an ape-like background leads to the first steps in the development of a mind capable of culture.

The male as hunter would necessarily have to use tools as Dart has pointed out for the australopithecines. Since the chimpanzee shows the rudiments of stick use and of the thrown missile, the natural variability upon which selection can act may be presumed to have been present in the protohominids. The effects of this selection pressure need not be further stressed here since they are familiar items in the literature of this topic since the time of Darwin as explained above. It is perhaps well to point out though, that in the integrated family unit the use of fire and tools in the domestic situation by the female would have survival value. Intelligence, therefore, need not have arisen exclusively as a male perogative!

Another important factor in the development of intelligence is the role of communication by speech which would be favored in an integrated family. Since the sphere of operation of the male and female are separated in space, communication by which specific information concerning items not immediately present such as the nature of the kill, spoors, weapons or fire-use, would be highly valuable. A selection pressure can therefore be visualized operating to activate language at one of its lowest levels, the use of word symbols for objects not immediately present. Such a pressure does not operate to any considerable extent in the lives of group living primates today since each animal is there a self-sufficient economic unit. There is little for them to communicate about except things present such as food or enemies. Such communication is sufficiently accomplished by the attention-attracting and emotion-arousing cries common to many social animals. Thus the alarm cry of apes is an emotional outburst that is highly effective in arousing other members to action against an aggressor. Köhler's account of the attack upon him following the alarm expressed by one of his

subjects is an illustration of this. Such a cry need have none of the ideational content of true speech. The social organization of contemporary apes therefore does not favor a selection pressure in the direction of language development.

It may of course, be objected that if this theory is valid the selection pressure for the development of language would operate for the wolf as for the protohominid. The failure of speech to develop in the wolf is, however, not surprising since the wolf's mentality is not well enough developed to provide the basis for selection. In short it is only after the level of mentality characteristic of the ape is reached where some ideational behavior is already present, that the shift to an integrated family will result in a development of language.

In the evolution of an integrated family economy there must have been some mechanism by which the male aggressiveness and dominance as found in other primates was mitigated to permit the male to become closely associated with the female in the common endeavor of family raising. I suggest that the dominance modification phenomenon as discussed above in the chimpanzee is the basis of such a change. The sexual relation produces a bond of cooperation during estrus in the chimpanzee (Yerkes, 1939). Since the female chimpanzee is in estrus about one third of her cycle this bond is of longer duration here than in lower mammals generally, which have a shorter estrus. In addition in the primates there is some use of sexual behaviors, especially sexual presentation, throughout the cycle as a means of mitigating aggression of a dominant animal. In an integrated family economy an extension of these tendencies would tend to tie the male and female together socially in a highly advantageous way. There would therefore be a strong selective factor making for the diffusion of sexual activity over the entire menstrual period and therefore a loss of the distinctive estrus phase. The development of a diffuse sexual activity between male and female as a type of socializing behavior would further be favored by ventral copulation which makes sexual play more feasible. Thus some of the distinctive characteristics of human sexuality, absence of estrus, relatively extended sexual activity and sexual play can be visualized as a part of the behavioral adaptation to the integrated family.

In lower mammals sexual behavior is predominantly under endocrine control operating through lower nervous centers. In higher mammals more cortical control independent of endocrines appears, particularly in the male. In the human this trend is strikingly accelerated, both male and female showing predominance of cortical rather than endocrine control of sexual behavior (Beach, 1948; Ford and Beach, 1951). It is here suggested that this shift in mechanism is part of the behavioral shift whereby sexual behavior became part of the socializing mechanism of the higher mammals particularly canines and primates. That this shift then is greatly advanced in the human may be one of the results of the selection pressure consequent upon the sociology of the hunting protohominid. Cortical expansion in the human is thus related to sexual behavior control as well as to the intellec-

tual functions. The mode of that sexual control was however quite different than that suggested by Chance and Mead as discussed in the introduction.

The suggestion of Bartholomew and Birdsell (1953) that the australopithecines male may have used tools in sexual competition need not necessarily be accepted. These authors have developed a concept of family organization in the australopithecines very similar to the one proposed here. In such a relation the sexual dimorphism shown by australopithecines may well be related to the differentiation of male and female function with respect to hunting rather than to intrasexual competition. The failure of the canines to enlarge or show dimorphism in the human line in spite of considerable dimorphism in size is consistent with both hypotheses. But the concept of a high level of male intrasexual competition is not consistent with the hypothesis of the integrated family theory proposed here. It may be that the very multiplicity of factors operating in this connection in human evolution accounts for the plasticity cultural man shows in regard to male-male and male-female relations (Mead, 1949).

The selection pressure favoring integration of the male may also have operated on the incipient language development discussed above. In apes we find that an intense devotion to grooming activity serves as a socializing factor between animals in a group. In human groups it is a common observation that speech, mere talking for the sake of talking in gossip and chit-chat serves a similar socializing function. The selection pressure for the integration of members of the family thus favors the development of speech as a socializing technique and helps account for the babbling of the human infant in contrast with the silence of the chimpanzee child (Hayes, 1951). That this socializing function of speech is correlated with the loss of body hair and much, though not all, of the drive for grooming is obvious but whether they are related as cause and effect or merely as concomitants is not clear.

The social organization of the integrated family visualized above would also shift selective pressures with respect to the development of the offspring. For one thing we might anticipate a relaxation of any pressure for rapid maturation. When the mother becomes permanently domiciled, there is no constant migration with which the young must keep up as there is in food-gathering primates. More important, however, than the relaxation of selection pressure for rapid maturation is a positive selection pressure postponing sexual maturation. Such a pressure would be another consequence of the socialization of the male for the following reason.

In baboon and macaque societies the in-group dominant males drive off the young males as they mature. These then tend to form bachelor groups ever alert for the opportunity of breaking into the mixed groups. The competition between the older in-group males and their male offspring is a disruptive factor in primate society and helps prevent the socialization of the primate males. One way to reduce this disruption is by a delay in attaining maturity. Such a delay would have the further advantage that it would permit a longer period for learning on the part of the young. The im-

portance of an extended period of learning can be appreciated better if it is kept in mind that a complex economy involving hunting, food-gathering and domestic activity requires very different activities in different seasons. Several yearly cycles experienced when the youth is far enough developed to take part in these activities would be most directly favorable for the required learning. This applies to the female as well as the male. The inefficiency in maternal behavior of the primiparous chimpanzee is a case in point (Yerkes and Tomlin, 1935). In a permanent domicile as visualized in the integrated family ecology the learning of maternal behavior by the adolescent female would clearly be favored. It may be pointed out that occasionally daughter coyotes den with their mothers so that the possibility of this kind of learning exists in canines (Jackson, 1951).

Admittedly these inferences as to the sociology of fossil man are speculative. Such speculation based on comparative behavior, however, need not be less reliable than that based on comparative anatomy. Both are necessary for the development of an understanding of the evolution and biology of man. The theory offered above attempts to provide the necessary basis in natural selection for explaining the transition of the evolving human organism from the non-cultural anthropoid level to the lowest cultural level.

It is not necessarily implied that the full range of mental capacity as we find it demonstrated in contemporary man is to be explained on this basis. Indeed the data of comparative behavior and the reasoning based upon it as discussed above make it evident that learning abilities and cooperative activities are related to specific functions in the ecology of the animal. Animals do not seem to develop behaviors that go beyond the functional requirements of their mode of life any more than they develop structures or physiological capacities beyond these requirements. This is a basic concept in our understanding of natural selection.

On this basis we expect selection pressure to push language development only to the point where it serves a function of identification of concrete objects and of socialization but not to the level of its use in abstract thought. Similarly the evolution of cooperative behavior can be explained to the point where it permits a degree of stabilization of the male into the family and pack but no further. In this view the origin of abstract thought, for example mathematical reasoning and of truly ethical behavior, for example the golden rule, are not explicable in the biological terms developed here. I do not propose to elaborate this conception of the limitation of the biological explanation. I mention it because an understanding of the biology of the transition from the non-cultural to the cultural level must include a comprehension of the limits of any theory as well as of its effectiveness.

## SUMMARY

A theory is sought to account for the evolution of the features of human mentality that make culture accumulation possible. Such a theory must suggest not only the selection pressures involved but also the basis in be-

havior of the protohominid upon which selection operated to lead to man's unique mental equipment. The following theory, based upon recent advances in our knowledge of the paleontology of man, of anthropoid psychology, and of the social behavior of vertebrates is offered. The protohominid lived in integrated family units essentially monogamous with a stable domicile. The male specialized as hunter, the female as domestic. A detailed consideration of the selection pressures operative in such an economy reveal the following modifications of the basic anthropoid adaptations; 1. increased intelligence in tool and fire use on the part of both male and female; 2. the beginning of language development; 3. social integration of the male and female through diffuse sexual behavior with increased cortical control; and 4. extension of the developmental period and the integration of the young males as well as females into the group. These biological factors are held to account for only the first steps toward a culture-capable organism.

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# CYTOPLASMIC EXCHANGE WITHOUT GAMETIC COPULATION IN THE WATER MOLD *BLASTOCLADIELLA EMERSONII**

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In a newly described species of Blastocladiella, *B. emersonii* (Cantino and Hyatt, 1953a), spores derived from resistant sporangia (R.S.) give rise to populations consisting of three different kinds of plants; they have been referred to as orange (O), ordinary colorless (OC), and late colorless (LC) plants. They were easily distinguished from one another by (1), their color, (2), their rate of growth, (3), the method and duration of motility, the viability, and the size of swarmers derived therefrom, and (4), the potentialities of R.S. plants produced by these same swarmers. Extensive examinations led to the conclusion, corroborated many times since then, that conventional gametic fusions such as those exhibited by *B. variabilis* (Harder and Sörgel, 1938) and *B. stubenii* (Stuben, 1939) and by the related genus *Allomyces* (Kniep, 1929; see also Emerson, 1941) never occurred between swarmers of orange plants and those of colorless plants. On the other hand, clones derived from the three types of plants displayed great internal variability in behavior patterns which were subject to quantitative and qualitative modification by selection and changes in the environment. For these and other reasons, it was postulated that the incidence and behavior of orange, ordinary colorless, and late colorless plants was controlled by a cytoplasmic factor.

Recently, a mutant strain (BEM) was isolated from cultures of *B. emersonii*; all the plants in a population of the mutant are orange, and they behave in most respects like the orange plants which appear in populations of the wild type (Cantino and Hyatt, 1953b). The motile swarmers of the mutant, like those of the wild type, *B. emersonii*, are also incapable of syngamy when mixed with swarmers derived from colorless plants.

In spite of the lack of obvious gametic copulation, however, orange swarmers do display a peculiar mode of behavior with their colorless counterparts. When observed in drops of water on blocks of plain agar, for instance, orange swarmers (either from the mutant or from the orange plants of *B. emersonii*) often approach and make contact with flagellate swarmers from colorless plants which have just settled down and become sluggish and quiescent, or which have settled, retracted their flagella, and in some instances even formed a minute germ tube. Having contacted such a partner, the orange swarmer crawls around it, very closely appressed and with intense pseudopodial activity as if in preparation for fusion. Meanwhile, the clearly visible flagellum undulates back and forth in constant motion.

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The process may involve only one or two extremely slow, amoeboid, "encirclements," but more generally, activity continues for 5–10 minutes with a maximum of ca. 20 minutes. The orange swarmer then moves away, often to repeat its gyrations with another. The quiescent or stationary swarmer which remains behind may then be approached successively by a second, a third, etc., orange swarmer, all of which, in turn, display such behavior once again. Just before breaking contact, the orange swarmer usually performs a series of discrete, jerky movements (as if it were trying to dislodge itself); its movements are quite reminiscent of the tugging and struggling displayed by a mitospore of Allomyces whose flagellum is trapped in the exit pore of a sporangium by another spore emerging behind it. However, the flagellum of the orange swarmer, as well as that of the partner if it, too, is still flagellated, is clearly free and there is no *visible* bridge between the two bodies when they are viewed *in vivo* under these conditions at magnifications of ca. 400 diameters (a bridge, however, *is* visible in fixed and stained preparations; see below). These peculiar gesticulations have never been observed to result in conventional sexual fusions.

Although such behavior is not known to occur among the motile cells of other aquatic fungi, it is strikingly suggestive of the manner in which the larger cells of Paramecium become laterally applied and then conjugate through overlapping cones for a rather brief period, undergo reciprocal nuclear (and sometimes cytoplasmic) exchange, and finally separate again as exconjugants. If something of this sort were, indeed, occurring in Blastocladiella, it seemed to us that it might be possible to verify such an exchange in much the same way that Lederberg and Tatum (1946) initiated their successful searches for genetic recombination in bacteria.

## METHODS

*B. emersonii* was grown on medium PYG½B (peptone, 1.25 g.; yeast extract, 1.25 g.; glucose, 3 g.; agar, 20 g.; in 1 liter of $10^{-2}$ M $NaHCO_3$), whereon separate, well-defined, viable resistant sporangial plants are produced; the generation time is ca. 4½ days at 20 C (Cantino, 1952). Spores derived from resistant sporangia were streaked on medium PYG (as above, but lacking bicarbonate), on which populations of thin-walled, colorless plants are produced; the generation time for such plants (OC types) is ca. 30–36 hours at 20 C (Cantino and Hyatt, 1953a). Blocks of agar bearing 20–50 of the latter were immersed in 10 ml. of water, and the suspensions of motile swarmers subsequently discharged from them were used in the ensuing search for an exchange phenomenon.

The mutant, BEM, was grown on medium PYG at 20 C, whereon orange plants are first produced which then give rise to orange clusters (clones); the latter gradually increase in diameter as the peripheral plants, in particular, discharge swarmers all of which remain localized in the immediate area and some of which are viable and produce new generations of orange plants once again. Blocks of agar bearing 3–4 of these clusters were immersed in 10 ml. of water, and the suspensions of motile swarmers dis-

charged from them were also used for the crossing experiments. All cultures were incubated at 20 C.

## RESULTS

*Experiment 1.* 0.05 ml. of the swarmer suspension of *B. emersonii* and 0.05 ml. of water were mixed together on each of five circular areas, ca. 3 cm. diameter, on the surface of medium PYG½B in a Petri dish; five such dishes were prepared. Five new cultures were then set up in similar fashion except that swarmers from the mutant were used instead of those from *B. emersonii*. Finally, 0.05 ml. each of both *B. emersonii* and mutant suspensions were mixed together on a third set of plates of PYG½B. Media were freshly prepared to ensure that rapid reabsorption of water would not result in decreased viability (Cantino, 1951); thus, all swarmers were free to move about in the surface film for some hours. Replicate experiments were slightly modified so that nine aliquots of the suspensions, instead of five, were transferred to each plate.

On PYG½B, mutant swarmers do not respond to bicarbonate and hence cannot produce R.S. plants, presumably because of a deficiency in alpha-ketoglutarate oxidase and aconitase (Cantino and Hyatt, 1953c). But, from the one per cent or less of these swarmers which are viable (Cantino and Hyatt, 1953b), there are derived populations of: (1), immature plants at various stages of development, none of which have yet laid down detectable amounts of orange pigment (gamma-carotene; Cantino and Hyatt, 1953b) and all of which cease growth prematurely. (2), mature orange plants which have not discharged swarmers. (3), plants bearing empty, terminal, spore sacs from which thousands of non-viable, orange swarmers have been discharged on to the surface of the agar immediately surrounding the parent plant; all swarmers rapidly disintegrate and liberate enough carotene to give the area a visibly orange tint. (4), plants bearing empty, terminal, spore sacs around which from one to perhaps two hundred of the several thousand swarmers have started growing; any of these which reach maturity become orange once again.

On this same medium, swarmers from *B. emersonii* produce (Cantino and Hyatt, 1953a): (1), individual plants bearing terminal, thick-walled, brown, pitted resistant sporangia. (2), immature colorless plants which cease growth prematurely. (3), thin-walled colorless plants which discharge swarmers on to the agar adjacent to the parent plant; in some, all swarmers are non-viable, whereas in others, a few to many of them are viable and develop into resistant-sporangial plants which impart a brown color to the newly formed clusters. (4), occasionally, orange plants that discharge swarmers, most of which are non-viable but some of which may develop into resistant-sporangial plants and, hence, produce brown clusters.

In this first experiment, therefore, total and differential counts were made of the various types of plants obtained on each plate. It was then determined if the populations resulting from the "cross" differed from those which would have been expected from the analysis of populations

found on the control plates inoculated with the mutant and *B. emersonii*, respectively.

In populations obtained from the "cross" (table 1), (1), the per cent non-viable plants (e.g., those which cease growth prematurely or which discharge all non-viable swarmers) and the per cent orange progeny were appreciably higher, and (2), the per cent individual resistant sporangial plants as well as the per cent clusters of resistant sporangial plants was appreciably lower than the combined values for the controls.

It was therefore essential to establish that the apparent loss of characteristics associated with *B. emersonii* and the corresponding gain in

TABLE 1

DIFFERENTIAL COUNTS AMONG POPULATIONS DERIVED FROM *B. emersonii*, BEM, AND THE CROSS *B. emersonii* × BEM; ON MEDIUM PYG½B.

| | Swarmers from: | A | B | C | D | E | F | Total |
|---|---|---|---|---|---|---|---|---|
| Exp. 1a | BEM | 72 | 243 | 16 | 37 | 0 | 0 | 368 |
| | B. emersonii | 22 | 169 | 0 | 0 | 403 | 26 | 620 |
| | BEM × B. emersonii | 90 | 433 | 4 | 56 | 251 | 14 | 848 |
| Exp. 1b | BEM | 426 | 409 | 173 | 364 | 0 | 0 | 1372 |
| | B. emersonii | 18 | 208 | 0 | 4 | 3205 | 261 | 3696 |
| | BEM × B. emersonii | 747 | 609 | 211 | 777 | 2910 | 50 | 5304 |
| Exp. 1c | BEM | 617 | 589 | 99 | 234 | 0 | 0 | 1539 |
| | B. emersonii | 135 | 723 | 0 | 0 | 1362 | 34 | 2254 |
| | BEM × B. emersonii | 690 | 1388 | 77 | 352 | 968 | 9 | 3484 |

A—Plants which discharged all non-viable swarmers
B—Plants which ceased growth prematurely
C—Clusters of orange plants
D—Individual orange plants
E—Individual R.S. plants
F—Clusters of R.S. plants

Derivations from above data:

| | Percent non-viable plants (A and B) | | | Per cent orange plants and orange clusters (C and D*) | | | Per cent R.S. plants (E) | | | Per cent R.S. clusters (F) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | B. e. + BEM | B. e. × BEM | Diff. | B. e. + BEM | B. e. × BEM | Diff. | B. e. + BEM | B. e. × BEM | Diff. | B. e. + BEM | B. e. × BEM | Diff. |
| Exp. 1a | 51.2 | 61.7 | +10.5 | 5.4 | 7.1 | +1.7 | 40.9 | 29.5 | −11.4 | 2.6 | 1.7 | −0.9 |
| Exp. 1b | 20.9 | 25.5 | + 4.6 | 10.7 | 18.5 | +7.8 | 63.2 | 54.5 | − 8.7 | 5.2 | 0.9 | −4.3 |
| Exp. 1c | 54.5 | 59.6 | + 5.1 | 8.8 | 12.3 | +3.5 | 35.9 | 27.8 | − 8.1 | 0.9 | 0.3 | −0.6 |

*All single, undischarged, orange plants are included here because the orange color is a characteristic associated primarily with the mutant. Actually, these plants also represent non-viable thalli which ceased growth after the pigment had been formed and which, therefore, did not discharge swarmers. Thus, if they are also included in the total non-viable counts (first column), the increase in "per cent non-viable plants" is further accentuated, as follows: Exp. 1a, 55 vs. 68 per cent; change, +13 per cent. Exp. 1b, 28 vs. 40 per cent; change, +12 per cent. Exp. 1c, 61 vs. 70 per cent; change, +9 per cent.

characteristics associated with the mutant was not due to the production of some sort of a toxic, diffusible substance by the mutant. Thus, thin (¼-½ cm. layers) of PYG½B were inoculated with the mutant as before, and the inoculated areas were then marked. At various intervals thereafter over a 5-day period, the discs of solidified media in the dishes were flipped over aseptically, and swarmers from *B. emersonii* were placed on the surfaces directly over the populations of the mutant which were now on the bottom. The subsequent counts of such populations of *B. emersonii* were practically identical to those obtained for control plates on which the mutant had not been grown. It seemed unlikely, therefore, that the cause of the phenomenon could be ascribed to the production of reasonably stable, diffusible, metabolic products by the mutant. It must be admitted, of course, that the production of a highly labile substance with a short half-life might not be detected by this technique, but the probability that this would have happened seems rather remote.

Finally, a careful microscopic examination of the populations derived from the "cross" also revealed the presence of a few resistant-sporangial plants which were distinctly different from the usual types and which were never found in populations derived exclusively from *B. emersonii*. Although these resistant sporangia were thick-walled and pitted, the wall pigment did not appear normal and homogeneous; this was due to the presence of small, granular areas, apparently just beneath the wall within the protoplast, whose color was an indubitable *reddish-orange*! (like the color of the mutant strain, due to its high content of gamma-carotene). All such resistant sporangia which have been tested failed to germinate when placed in water in the usual way; they were apparently non-viable, like most plants in a population of the mutant strain.

The foregoing results strongly suggested that cytoplasmic exchange had occurred between the mutant and wild type cultures of Blastocladiella.

*Experiment 2.* 0.2 ml. of a suspension of swarmers from *B. emersonii* and 0.2 ml. of water were mixed together on each of ten plates of synthetic medium B (phosphate, $MgSO_4$, glutamate, glucose, methionine, and thiamine; Barner and Cantino, 1952) in which all organic constituents were Seitz filtered separately. Similarly, 0.2 ml. of a suspension of mutant swarmers and 0.2 ml. of water were transferred to another ten plates of medium B. The inoculum, which was made to cover as small an area as possible, was not disturbed for five minutes; it was then spread out as a thin film over a wide area by tilting the dishes appropriately. Finally, 0.2 ml. each of the suspensions of *B. emersonii* and BEM were mixed together on a third set of plates. In two of the latter, the suspension was spread out after the initial five-minute period as before; of the remaining plates, two were so treated only after 45, two after 65, two after 80, and two after 95 minutes. The media were freshly prepared, and hence there was no appreciable absorption of the water by the agar during the 95-minute period.

*B. emersonii* grows well in liquid medium B; in this same medium, the mutant exhibits no detectable growth. Total counts of individual, first-

TABLE 2

TOTAL COUNTS OF POPULATIONS DERIVED FROM *B. emersonii*, BEM, AND THE CROSS *B. emersonii* × BEM; ON SYNTHETIC MEDIUM B.

| Swarmers from: | Total counts | | |
|---|---|---|---|
| | Exp. 2a | Exp. 2b | Exp. 2c |
| B. emersonii | 1563 | 2875 | 1244 |
| BEM* | 39 | 151 | 4 |
| B. emersonii × BEM | 10020 | 5063 | 2930 |

* On media such as PYG, the same aliquots of BEM swarmers would have yielded ca. 1400, 5200, and 140 plants, respectively. This, in turn, would represent only about one per cent of the total number of swarmers actually present (namely, 140,000, 520,000, and 14,000, respectively) since ca. 99 per cent of them are non-viable on PYG (Cantino and Hyatt, 1953b and references therein).

generation plants were therefore made on the plates of solidified medium B described above. The number of plants in populations resulting from the "cross" was about six times the combined count of the separate populations of *B. emersonii* and the mutant strain; in replicate experiments, wherein the mutant and *B. emersonii* swarmers were exposed to one another for only 15 minutes before spreading, a ca. 2- and 2½-fold increase in total count occurred (table 2).

Perhaps even more important, however, was the fact (figure 1) that the number of plants in populations derived from the "cross" (experiment 2a in

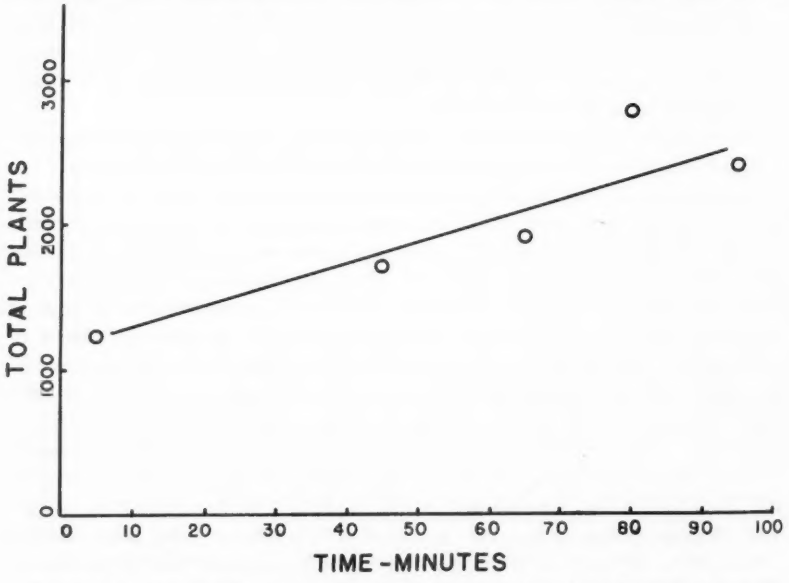


FIGURE 1. The relation between (1), the length of time that mixtures of swarmers from *B. emersonii* and the mutant remained in close proximity, and (2), the total counts of populations subsequently derived therefrom (Experiment 2a).

table 2) was a function of the length of time (5, 45, 65, 80, and 95 minutes) that the swarmers from the mutant and *B. emersonii* remained in relatively close proximity before being separated from one another in the thin film over the agar surface. Thus, through the use of nutritional criteria for differential "tagging," and with the introduction of the time factor, the results strongly suggested once again that some kind of exchange had occurred.

*Experiment 3.* Finally, because the evidence from the foregoing experiments was not only consistent with, but lent significance to, the initial *in vivo* observations of the behavior of orange and colorless swarmers with one another, attempts were made to obtain further direct cytological evidence for the phenomenon.

Equal aliquots (ca. 0.05 ml.) of swarmer suspensions of *B. emersonii* and the mutant were mixed together on microscope slides; aliquots of each suspension were also transferred to separate slides as controls. Fifteen minutes later, swarmers were exposed to the fumes of one per cent osmic acid for 90 seconds and were then stained with 0.05 to 0.10 ml. of 0.05 per cent aq. crystal violet. Differential counts were made for approximately half the population on each slide; representative results are presented in table 3.

TABLE 3

DIFFERENTIAL COUNTS OF SWARMER SUSPENSIONS, FIXED AND STAINED, AND DERIVED FROM *B. emersonii*, BEM, AND THE CROSS *B. emersonii* × BEM.

| Swarmers from: | Total counts* | |
|---|---|---|
| | Single uniflagellate swarmers | Paired uniflagellate swarmers |
| B. emersonii | 55 | 0 |
| BEM | 77 | 0 |
| B. emersonii × BEM | 118 | 6 |

*In replicate experiments, the ratio of paired uniflagellate swarmers to single uniflagellate swarmers was 7/270, 5/132, 8/70, and 4/64; once again, paired swarmers were never found on the control slides.

The slides of *B. emersonii* and those of the mutant invariably contained only well-defined, well-separated, uniflagellate swarmers and a few biflagellate swarmers which apparently resulted from incomplete cleavage. On the slides bearing both *B. emersonii* and the mutant, however, invariably there was found, in addition to the uniflagellate and the same proportion of biflagellate cells, a small number of paired swarmers. This number of pairs appeared to be, primarily, a function of the population density of the BEM. One member of the pair was almost always small and uniflagellate (the mutant), while the other was larger and sometimes did not possess a flagellum (the *B. emersonii*). These experiments made possible the use of magnifications appreciably higher than those employed in the initial *in vivo* observations; thus, it was discovered that on each slide containing both *B. emersonii* and the mutant, many of the paired swarmers *were clearly connected by a cytoplasmic bridge*! (figures 2 and 3). The relatively large and

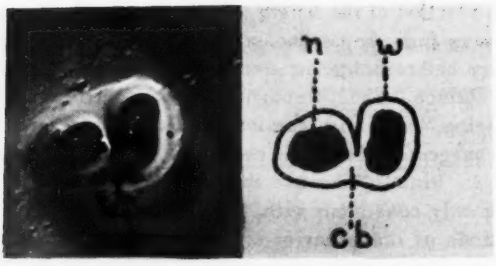


FIGURE 2. A pair of swarmers connected by a well-defined cytoplasmic bridge (cb; and see arrow), the smaller one (left) having been derived from the mutant and the larger one (right) from *B. emersonii*. With the photographic techniques used (G. E. #AH4 U. V. lamp and E. Kodak #58B green filter), the usually-distinct nuclei and associated nuclear caps could not be differentiated from one another. They therefore appear blended together and fused in the form of large, centrally-located, uniformly and deeply stained, massive bodies (n) which occupy a large proportion of the total volume of the swarmers. The cell wall of the swarmers is indicated at (w). Under the circumstances, also, flagella could not be brought into focus. × 1400.

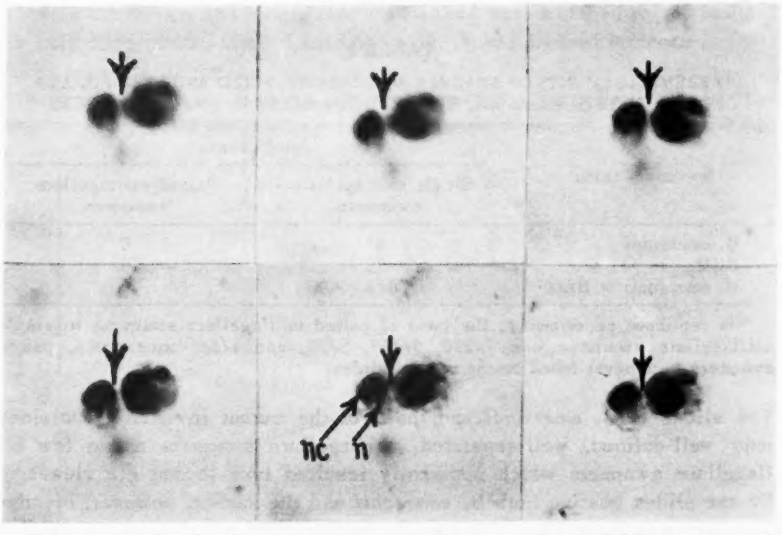


FIGURE 3. A pair of swarmers connected by a cytoplasmic bridge (see arrow), the smaller one having been derived from the mutant and the larger one from *B. emersonii*. Photographs (upper left to lower right) were taken at progressively higher optical levels, roughly one to two microns apart, in order to delimit as clearly as possible the thickness and the orientation of the cytoplasmic connection. The individual nuclei (n) and their associated nuclear caps (nc) are easily differentiated at certain optical levels. Both figures 2 and 3 were prepared from aqueous suspensions of swarmers killed with fumes of osmic acid. However, whereas the cells in figure 2 were stained with crystal violet as outlined in the context, those in figure 3 were mounted directly in a permanent mounting medium, developed and kindly supplied to us by Dr. Conway Zirkle, whose composition was as follows: lactate, 25 g., phenol, 40 g., glycerine, 20 g., water, 15 g., and lacmoid, 1 g.

obvious nuclei and nuclear caps retained their individuality within each swarmer; if, then, the bridge were indeed functional, these observations implicated a cytoplasmic rather than a nuclear exchange mechanism.

## DISCUSSION AND SUMMARY

From the data now available (cf. summary in table 4), it would be premature to draw extensive conclusions about the quantitative nature of the exchange which apparently occurs when swarmers from colorless plants of *B. emersonii* are mixed with those from orange plants of its mutant strain (and, presumably, with those from orange plants of *B. emersonii* which be-

TABLE 4

A SUMMARY OF THE RESULTS OBTAINED FROM THE CROSS B. *emersonii* × BEM.

| Experiment | Results | | |
|---|---|---|---|
| | B. emersonii | BEM | B. emersonii × BEM |
| Observations of swarmers in-vivo | No pairing | No pairing | Pairing of small BEM swarmers with larger B. emersonii swarmers. |
| Observations of swarmers fixed and stained | No pairs | No pairs | Numerous pairs consisting of single BEM swarmer and a larger B. emersonii swarmer, the two often connected by a cytoplasmic bridge. |
| Observations of populations derived from swarmers streaked on medium PYG½B | Normal population | Normal population | "Modified" population exhibiting an increase in mutant characters (orange and non-viable plants) and a decrease in wild type characters (R.S. plants and R.S. clusters). |
| Observations of populations derived from swarmers streaked on synthetic medium B. | Normal population | Normal population | "Modified" population exhibiting an increase in the wild type characters (increase in viability, and thus, total count on the synthetic medium). |

have similarly *in vivo*). It can only be concluded (table 3) that a certain minimum per cent of all mutant swarmers may pair up and undergo some sort of exchange with swarmers from *B. emersonii*. But, it must be remembered, judging from our *in vivo* observations, that such mutant swarmers can then repeat the process with a second, a third, and etc. swarmer from *B. emersonii*, and that any one swarmer of *B. emersonii* may be successively approached, in turn, by more than one swarmer from the mutant. Hence, it is as yet impossible to calculate either an average or a maximum value for per cent exchange; it can only be surmised that the latter may be surprisingly high.

In this connection, it may appear that the quantitative results (3–10 per cent pairing) derived from the foregoing cytological observations are at variance with those presented in table 2, wherein the "cross," *B. emersonii* × mutant appeared to yield a 2–6 fold increase in population size as compared to the combined totals of the controls. But the total and differential counts in table 3 were made on *all visible* swarmers, whereas of these very same swarmers, only about one per cent of those derived from the mutant are sufficiently viable on rich media such as PYG (Cantino and Hyatt, 1953b) to germinate and produce plants which can be counted, and far fewer can do so on the synthetic medium B (footnote, table 2). Thus, in experiment 2, any number of the thousands of remaining non-viable ones may have undergone exchange with one or more swarmers from *B. emersonii* with a concomitant inheritance of factors for increased viability.

Finally, although the "crossing" experiments were designed solely to corroborate and amplify our *in vivo* observations, the results derived therefrom do lend themselves to some speculation regarding the qualitative nature of the exchange. From the data in table 1, it is clear that there has occurred among populations resulting from the cross on medium PYG, (1), an increase in the incidence of characters that are associated with the mutant (e.g., low viability and synthesis of carotene), and (2), a decrease in the incidence of characters that are associated with the wild types (e.g., high viability and production of brown resistant sporangia). The results suggest that transfer of cytoplasmic factors may be mostly unilateral, either from the mutant to *B. emersonii* or *vice versa*. From the data in table 2, it is evident that there has occurred among populations resulting from the "cross," an increase in the incidence of a character associated with the wild type (e.g., the potentialities for growth on the synthetic medium). These results, too, suggest a more or less unilateral transfer of "factor(s)" which permit the mutant strain to develop on the highly selective synthetic medium B.

We conclude, therefore, that whereas ordinary gametic copulation does not occur in the aquatic Phycomycete, *Blastocladiella emersonii*, its motile swarmers do exhibit a new atypical mode of behavior which appears to result in unilateral transfer of certain cytoplasmic materials.

Finally, we wish to call attention to certain implications which result from the apparent demonstration of cytoplasmic exchange in *Blastocladiella:*

(1), It will further complicate, and at the same time facilitate investigations of the already inordinately complex life cycle of *B. emersonii*; it will certainly also play a major role in the distribution of our hypothetical cytoplasmic factor, gamma (Cantino and Hyatt, 1953a) which is believed to control certain behavior patterns in the fungus. In particular, the fact that the transfer of cytoplasmic materials between orange swarmers of the mutant and colorless swarmers of *B. emersonii* may induce the latter to become orange (and thus "male" plants) lends significant support to the conten-

tions by Emerson (1950) and Cantino and Hyatt (1953a) that the expression of "sex" in Blastocladiella is not genotypically controlled.[1]

(2), It may have a profound effect on the interpretation of genetic and physiologic investigations among the Chytridiales, other Blastocladiales, and perhaps even other aquatic fungi, in the same way that the cytoplasm has been shown to play a vital role in the behavior of Paramecium (e.g., Sonneborn, 1950), yeasts (Ephrussi, 1953), and other microorganisms. Although there is admittedly no reason to assume, at this time, that some similar sort of recombination does occur in aquatic Phycomycetes other than Blastocladiella, there is also little reason to assume that it does not.

(3), Finally, from the point of view of comparative biology, it reveals the existence of an exchange mechanism in a primitive plant which is akin to, although not necessarily identical with, that found in a primitive animal such as Paramecium.

ACKNOWLEDGEMENT

We are indebted to Miss Mildred T. Hyatt for her cooperation during part of this investigation.

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[1] Recent preliminary studies of wild type and mutant cells stained with the Nadi reagent (Dimethyl-p-phenylenediamine and alpha-naphthol) reveal the presence of many small, deep blue-black cytoplasmic particles in the former; such particles are generally absent in the latter. Earlier studies have already demonstrated the occurrence of ketoglutarate oxidase and aconitase in the wild type, where they are involved in the morphogenetic mechanism leading to the formation of a resistant sporangium; these enzymes are absent in the mutant strain, which has an extremely low viability, cannot produce resistant sporangia, and synthesizes large quantities of gamma carotene. Our *working hypothesis* is that these particles stainable with the Nadi reagent may correspond to our cytoplasmic factor *gamma*, that they carry the ketoglutarate oxidase and aconitase, but not isocitric dehydrogenase, succinic dehydrogenase, fumarase, malic dehydrogenase, and cytochrome oxidase, and finally that they are one of the factors involved in the kind of cytoplasmic exchange described herein. Thus, if the hypothesis is correct, "maleness" in the wild type *Blastocladiella emersonii* is determined not genotypically but, rather, (1), by the random distribution of cytoplasmic particles during formation of swarmers in individual plants, and (2), by the transfer of such particles *via* the temporary cytoplasmic bridge which is formed between orange and colorless swarmers before they germinate to produce new plants.

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# THE EFFECT OF YEAST ON PHOSPHORUS UPTAKE BY DROSOPHILA[1]

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## INTRODUCTION

The importance of yeasts in the nutrition of Drosophila has been recognized since the early work of Guyenot (1913), Northrup (1917) and Baumberger (1917, 1919). More recently the subject has been reopened from an evolutionary standpoint by Wagner (1944, 1949), da Cunha (1951), da Cunha, Dobzhansky and Sokoloff (1951), and El-Tabey Awab Shehata and Mrak (1952). El-Tabey Awab Shehata and Mrak (1951), while studying the passage of yeast through the gut of *Drosophila pseudoobscura*, showed that yeast RNA is completely extracted during the process. It would appear therefore that yeast is an important source of supply of phosphorus to the fly. This paper will describe striking differences in the uptake of labeled phosphorus by two related species of Drosophila feeding on different yeast species.

## MATERIAL AND METHODS

The insects used in these studies were wild type *Drosophila melanogaster* (Canton-Special) and *D. simulans* (South Africa). The stocks were obtained from Professor D. F. Poulson of Yale University. The yeast species used were: *Saccharomyces cerevisiae* from commercially available Fleischmann's bakers' yeast, *Candida albicans* obtained from the California Institute of Technology, and *Debaryomyces matruchoti* (NRRL Y-833), *Hansenula subpellicosa* (NRRL Y-1683) and *Schizosaccharomyces pombe* (NRRL Y-675) all originally obtained by Dr. V. T. Bowen of the Brookhaven Laboratory from Dr. L. J. Wickerham of the Northern Regional Research Laboratory, Peoria, Illinois. The non-pathogenic strain of *Candida albicans* previously referred to was isolated from a rotting fruit of *Opuntia lindheimeri* from Austin, Texas, by Dr. R. P. Wagner and is designated by him as strain Y-2.

The radioactivity of flies was measured with a restricted atmosphere proportional counter as described in a previous paper (King, 1953). Samples were counted while cemented with a drop of collodion solution (0.1 per cent collodion in 1 : 1 ether-alcohol) on flat copper discs (1 inch diameter), flat stainless steel discs (1 inch diameter), aluminum discs (1-1/4 inch diameter) or aluminum cups (1-1/4 inch diameter, 1/8 inch deep). Samples were counted for a sufficient period of time to give a net count of at least 5000, so that the standard deviation was less than two per cent. Ignitions were always made on stainless steel discs.

[1]Research carried out at Brookhaven National Laboratory under the auspices of the U. S. Atomic Energy Commission.

Labeled medium was obtained by adding $H_3P^{32}O_4$ to "standard" cornmeal, molasses, yeast, agar medium. The composition of this medium has been described in an earlier publication (King, 1953). The radiophosphorus used in all experiments was supplied by the Oak Ridge National Laboratory at Oak Ridge, Tennessee. The total phosphorus concentration of the medium was known (0.31 mg P/g), as well as the activity of the medium in microcuries per gram in each case. Added orthophosphate containing $P^{32}$ would be expected to come into equilibrium with larger or smaller fractions of the total phosphorus of the medium depending upon the extent to which orthophosphate is released accompanying breakdown of phosphorus compounds in the medium by various yeast species. Because of this, it is impossible to calculate absolute values for the phosphorus uptake of flies from a knowledge of their radioactivity. Consequently the data are of importance only from a relative standpoint and will be condensed as much as possible in the following presentation.

## EFFECT OF YEAST SPECIES

A study was made on the effect of various yeast genera on the average phosphorus uptake by adult *Drosophila melanogaster* and *D. simulans*. Each of five series of creamers containing propionic acid-free radioactive medium was inoculated with one of the five species of yeasts previously described. Propionic acid-free medium was used, since it had been noticed earlier that this anti-mold agent added to Drosophila medium at concentrations of 4 mg/g medium markedly inhibits growth of Hansenula and Debaryomyces. Paper towelling or Kleenex is often placed in fly cultures, but this procedure is always avoided, since it cuts down on phosphorus uptake and increases the variability in the radioactivity of flies. After a three days incubation at 30° C the surface of the medium in all creamers was covered with a thick layer of yeast. It should be noted parenthetically that previous work showed that males from a population of 25 contain twice as much radiophosphorus after feeding for one day as those from a population of 250, but phosphorus uptake for a given sex is identical whether the sexes are raised separately or together provided the population density remains the same. Phosphorus uptake was found to be the same in diffuse light or in total darkness. In this case 25 male and 25 female (2–4 day old) *D. simulans* or *D. melanogaster* were allowed to feed together on the food surface for one day at 25° C. The average activity of ten males or females was recorded. The 24 hour feeding period was too short to allow the flies to eat their way to the medium. Consequently their diet was primarily yeast. Comparison was made with data collected from flies fed on unyeasted propionic acid-free and propionic acid-containing medium having the same radioactivity and phosphorus content per gram as the medium of the other series.

The results are summarized in the bar graph of Fig. 1. All values are relative; the count given by the average *simulans* male on unyeasted, propionic acid-containing medium is taken as unity. Comparisons can be made

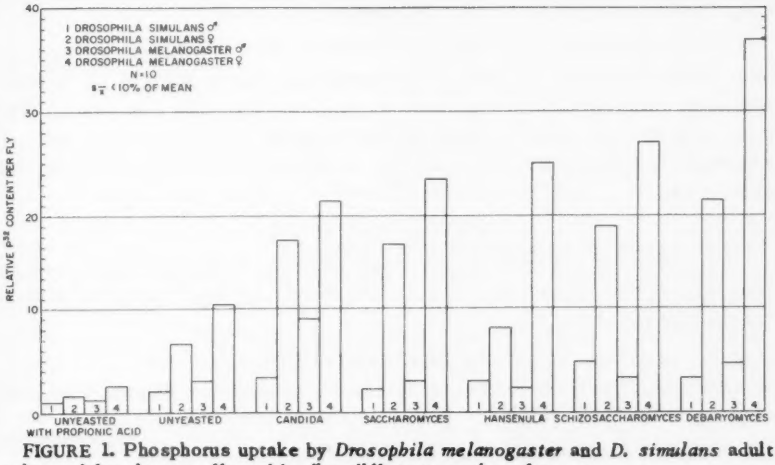


FIGURE 1. Phosphorus uptake by *Drosophila melanogaster* and *D. simulans* adult males and females as affected by five different species of yeast.

$$s_{\bar{x}} = \sqrt{\frac{\Sigma x^2 - \frac{(\Sigma x)^2}{n}}{n(n-1)}}$$

safely between males and females of the two Drosophila species on a given yeast, but not between flies on different yeast species. In all cases females take up more labeled phosphorus than males. All yeasts except Candida raise the *melanogaster* female/male ratio above the control value, while all yeasts except Hansenula raise the *simulans* female/male ratio above that of the control. Generally *melanogaster* and *simulans* males take up similar amounts of phosphorus. *Melanogaster* males are clearly superior, however, in the Candida series. *Melanogaster* females from all series are more radioactive than *simulans* females. Adult flies placed on yeast-free medium contaminate it with yeasts carried on their bodies. One would expect growth of contaminating yeast to be retarded on propionic acid-containing medium. Perhaps the difference in phosphorus uptake between flies on unyeasted, propionic acid-containing medium and those on unyeasted, propionic acid-free medium is due to ingestion of yeasts from small contaminating colonies in the second series.

It is concluded that the rate of phosphorus uptake of adult females of the Drosophila species tested is always faster than that of males. Flies feeding on yeast take up phosphorus at a faster rate than flies feeding on yeast-free medium. This increase in the rate of phosphorus uptake is generally greater in females than males. For a given yeast the increase in the rate of phosphorus uptake is sometimes strikingly different in the case of flies of the same sex belonging to different species.

SUMMARY

A study was made of the effect of five yeast species upon phosphorus uptake by adults of two species of Drosophila. It was found that phosphorus uptake by both species is increased on medium containing live yeast. The rate is increased more in females than in males. Phosphorus uptake is sometimes strikingly different for flies of different species feeding on the same yeast.

ACKNOWLEDGEMENTS

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# REPRODUCTIVE CAPACITY IN A PULMONATE SNAIL (*PHYSA GYRINA* SAY)

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Reproduction in *Physa gyrina* under natural conditions is directly related to temperature. Seasonal periodicity of oviposition disappears in laboratory reared individuals and the egg laying period begins when the snails have reached a certain morphological and physiological state of development. The study of reproduction in *P. gyrina* under laboratory conditions is more effective since there are several factors over which no control is possible in the field. Under experimental conditions all snails were fed the same diet throughout their life, light and temperature remained relatively constant for all individuals, and infection by larval trematodes was eliminated. In addition, it was possible to determine the length of life of individual snails, their fecundity and the effect of isolation upon reproduction. Data obtained from field collected individuals, although subject to obvious limitations, are compared to those obtained under experimental conditions whenever pertinent.

Because of the incomplete nature of the field data (such as the necessity of making estimations of duration of life span of snails in their natural habitat), the application of normal statistics was not advisable.

## FIELD COLLECTED *Physa gyrina*

The specimens studied were isolated within an hour or two after they were brought into the laboratory and checked daily for as long as they lived. Egg masses were removed as laid, the eggs counted and the stage of development of the embryos noted. Succeeding embryonic development within each mass was followed and the number failing to pass through the trochophore and/or post-trochophore stage, together with the number hatching was recorded.

Snails were collected from January through April when most of the population was sexually mature. At that time, oviposition in the field was inhibited by low temperature. The majority of the snails began to oviposit within 24 hours at room temperature. Initially the masses tended to contain the maximum number of eggs and two to five masses were produced each day. Number of eggs per mass and the number of masses progressively declined so that near the end of the ovipositing period, several days might elapse between the deposition of masses. A similar decrease in egg production has been reported in Ancylidae (Bondesen, 1950; Clapp, 1921), Planorbidae (Bondesen, 1950; Cole, 1935) and Lymnaeidae (Bailey, 1939; Noland and Carriker, 1946; Schodduyn, 1925; Seshaiya, 1927; Taki, 1931). No data pertinent to Physidae are available.

## LABORATORY REARED *Physa gyrina*

Since it is impossible to determine whether or not snails in the field had oviposited previous to collection, their reproductive capacity cannot be accurately established. This is possible only when individuals are kept under observation from birth.

*Physa gyrina* were reared from eggs laid by individuals collected in the field from two localities. The first is a series of small pools at the base of Argo Dam on the Huron River within the city limits of Ann Arbor, Michigan. The second locality at Scio is about six miles upstream from the former and is the site of a study on a field population of *P. gyrina* (DeWitt, in press). Both isolated and communally reared snails were selected at time of hatching and raised in constantly aerated water in glass containers. The purpose of aeration was not to supply dissolved oxygen to the snails (they use atmospheric oxygen which they obtain by frequent trips to the water's surface) but an attempt to oxidize metabolic wastes and thus prevent their accumulation. Production and development of eggs were treated as described for field collected snails. The pattern of oviposition for laboratory reared individuals is similar to that in field collected *P. gyrina.*

The $F_1$ generation of *P. gyrina,* from both Argo and Scio stock, reared in isolation was self-fertile. That parthenogenetic development did not occur was demonstrated by the formation of two polar bodies during maturation of the ova. Only one individual of the $F_2$ generation, raised in isolation or together, laid eggs which hatched to produce an $F_3$ generation. About 50 per cent of the $F_2$ generation failed to oviposit; eggs produced by the others, with the one exception noted, failed to pass through late cleavage.

## DISCUSSION

The ecological age concept of Bodenheimer (1938) provides a workable basis for denoting various periods within the life span of organisms. The life cycle is divided into three portions: period of development—time from laying of egg until individual produces gametes; period of reproduction—lasting throughout the overt reproductive cycle; period of post-reproduction —time from the end of reproduction to death. Studies on a field population of *Physa gyrina* (at Scio) agree with those on other animals (Allee et al, 1949); the period of development is longest and that of post-reproduction shortest. This does not apply to laboratory reared snails. In the stock obtained from Scio, the period of post-reproduction was greater than the total time required for development and reproductive activity. The developmental period of those reared from Argo stock was longest but reproduction was shortest.

In this species, the relative proportions of time occupied by the various periods of ecological age is related to the physical environment. A valid comparison can be made only between the field population studied at Scio and the individuals reared in the laboratory from Scio stock. There was no appreciable difference between the total life span in either case. Snails born in the field during the spring become sexually mature by late summer

or fall of that year but oviposition is mostly delayed until higher temperatures occur in the spring. Thus what appears to be a prolonged period of development is not in actuality since the period of reproduction is not instigated until environmental factors (namely temperature) become favorable. Therefore the periods of reproduction and post-reproduction are correspondingly reduced. Under constant environmental factors in the laboratory, the inhibiting effect of low temperature is removed and oviposition takes place when the gametes have matured.

In the Scio locality, *Physa gyrina* lives approximately 12 to 13 months. The mean life span of laboratory reared snails from both Argo and Scio stock was 14.5 months. Communally reared *P. gyrina* lived a longer (about eight per cent) time than those reared in isolation. Isolated snails had a longer period of development, were larger (shell length) at time of oviposition and laid 3.3 times as many eggs per snail than did those individuals reared together (table 1). Crabb (1929) postulated that the shortened

TABLE 1

MEAN LIFE HISTORY DATA

(Time is expressed in days. The figures in parentheses under the various periods represent the percentages of the total life span.)

| | Developmental period | Reproductive period | Post-reproductive period | Total life span | Shell length in mm. at oviposition | Number egg masses per snail | Number eggs per snail |
|---|---|---|---|---|---|---|---|
| Field collected | .... | 25 | 4 | .... | 12.8 | 22 | 653 |
| Reared in mass culture | 127 (29) | 94 (22) | 211 (49) | 432 | 9.7 | 18 | 253 |
| Reared in isolation | 169 (42) | 86 (22) | 143 (36) | 398 | 12.0 | 42 | 830 |

length of life of laboratory reared *Lymnaea stagnalis appressa* was due to a continuously high metabolic rate which is not present in snails in nature where seasonally controlled rest periods occur. I found no indication that rest periods are essential to the sequence of events within the life cycle of *P. gyrina* nor that lack of them shortened the life span. The shorter life span of isolated individuals may conceivably be accounted for by the greater amount of energy expended in egg production.

TABLE 2

SIZE, EGG PRODUCTION AND VIABILITY IN *Physa Gyrina* REARED IN ISOLATION

| Number of snails | Mean length of shell in mm. at oviposition | Mean number of eggs per snail | Viability in per cent |
|---|---|---|---|
| 3 | 8.2 | 64 | 30 |
| 6 | 12.6 | 859 | 60 |
| 3 | 14.2 | 1124 | 64 |

TABLE 3

SIZE, EGG PRODUCTION AND VIABILITY IN *Physa Gyrina* REARED IN MASS CULTURE

| Number of snails | Mean length of shell in mm. at oviposition | Mean number of eggs per snail | Viability in per cent |
|---|---|---|---|
| 8 | 7.0 | 46 | 76 |
| 6 | 9.0 | 245 | 67 |
| 6 | 9.5 | 272 | 91 |
| 2 | 13.0 | 450 | 57 |

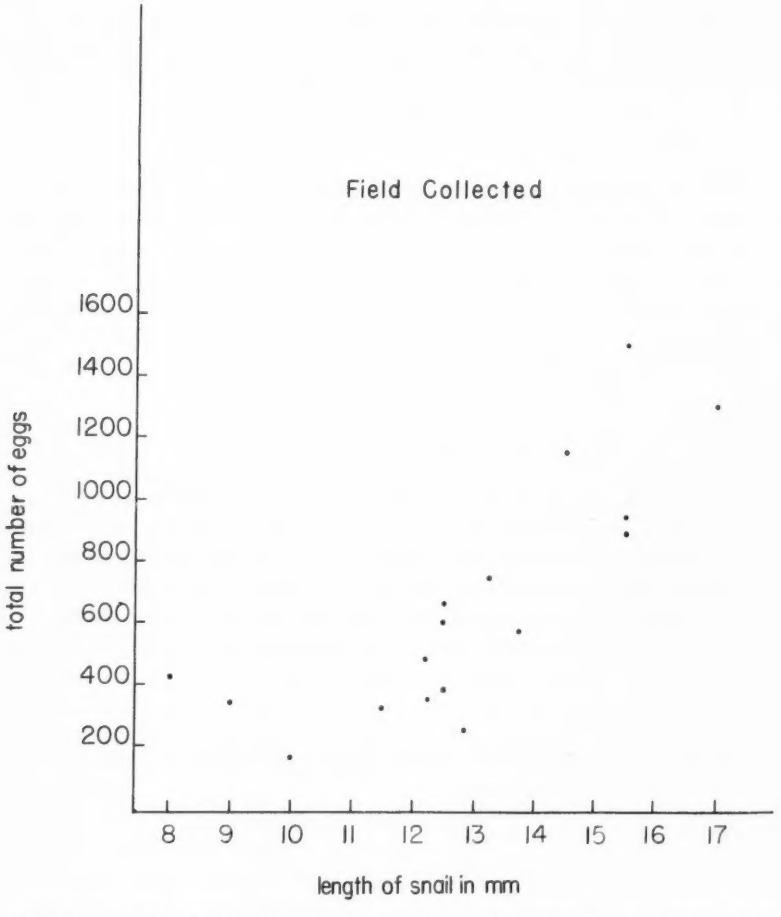


FIGURE 1. Correlation between size (shell length) and total egg production in field collected *Physa gyrina*.

A very definite relation exists between population density, size and egg production. As the number of snails reared together was increased, the size (shell length) at time of oviposition decreased and the mean number of eggs produced per snail decreased (tables 2 and 3). That time is not the sole factor in determining shell length is established by the periods of development. The same positive correlation between size and egg production exists for those snails collected in the field (fig. 1). The observations of Boycott et al (1930) and Noland and Carriker (1946) that snails raised in isolation undergo a longer developmental period than do communally reared snails is verified by this study on *P. gyrina* (table 1).

The percentage of hatching was greatest in eggs of field collected snails and lowest in those individuals reared in isolation (table 4). The drop in

TABLE 4

VIABILITY OF EGGS (MEAN PERCENTAGES)

| | Died as trochophore | Died as post-trochophore | Hatched |
|---|---|---|---|
| Collected in field | 7 | 4 | 89 |
| Reared in mass culture | 1 | 26 | 73 |
| Reared in isolation | 11 | 38 | 51 |

viability of eggs of isolated snails is not due to failure of the individual to fertilize its own eggs since all underwent cleavage; nor can it be accounted for by a simple genetic mechanism. A pre-imaginal mortality, as described for Drosophila, which varies directly with density of population (Winsor, 1937) does not appear to be a factor as the eggs of eight *P. gyrina* reared together had a higher viability than did those of six or two snails. There was no evidence that fertility increased or decreased with age of the parent.

Crabb (1927) stated that pond snails isolated in "ovo" laid the same number of eggs per snail as those individuals reared in mass culture. I found that isolated *P. gyrina* laid twice as many eggs per snail as two snails reared together; three times as many as six individuals and eighteen times as many as eight. Contrary to Crabb's report (1927), the percentage of hatching in these "virgin" eggs was considerably lower than in those eggs of snails which had an opportunity to cross-fertilize. That size (shell length) was not alone responsible for the greater fecundity of isolated snails is demonstrated by the fact that snails of a comparable size raised in mass culture produced a smaller number of eggs.

The ability to self-fertilize is of considerable value when, due to adverse environmental factors, the numbers of a population decline and chances for cross-fertilization decrease or an individual is transported to a new habitat lacking individuals of the same species. As has been

intimated, this species is probably not incapable of temporarily perpetuating itself, by selfing, under natural conditions. The failure to produce an $F_1$ generation in the laboratory may be due to an accumulative effect resulting from the lack of some vital factor.

ACKNOWLEDGMENT

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# LETTERS TO THE EDITORS

Correspondents alone are responsible for statements and opinions expressed. Letters are dated when received in the editorial office.

## ANIMAL BREEDING UNDER LYSENKO

In August, 1948, T. D. Lysenko became the overlord of the biological sciences in USSR. From that time on, no scientific periodical in U.S.S.R. has published articles dealing with research in proscribed fields, such as basic genetics. Whether such research is still going on under cover of other activities is impossible to tell. That the work in applied genetics has not been completely abandoned is, however, certain. A review article by Kushner gives an interesting insight both into the conditions under which the work on animal breeding is going on and into some of the results obtained.[1]

As an orthodox Lysenkoist, Kushner begins his article as follows: "The Soviet creative Darwinism, which grew and matured under the influence of the well-known works of I. V. Michurin and of the academician T. D. Lysenko in the field of plant breeding, has exerted powerful influence on many related biological and agricultural sciences, including the animal breeding. In the struggle with the Weismannist-Morganist reactionary-idealist theories of immutability and immortality of the so-called germ plasm, of incomprehensibility of the causes of hereditary variability, and of isolation of heredity from the environment, there has grown in our country, led by the Communist Party, an army of Michurinist animal breeders, who successfully realize the precept of their teacher: 'We cannot wait for gifts from nature; our task is to get them from nature. Michurin.' The accomplishments of the Michurinist animal breeders disprove quite conclusively the dogma of formal genetics that the genotype is independent of the environment, that valuable breeds cannot be created by hybridization, and that the offspring of hybrids continuously and unavoidably segregate." Any educated biologist could have told Professor Kushner that these "theories" and "dogma" exist only in his imagination, and that scientific genetics either never entertained them or has shown them to be invalid long ago.

Having successfully overcome the illusions of genetics, the Michurinist M. F. Ivanov discovered that the breeds to be created should be "adapted to local climatic, soil, nutritional, and economic conditions," and that this can be accomplished by careful selection of suitable individuals among hybrids between specialized foreign breeds and local breeds which possess an adaptedness to the local environments. He also discovered that, to avoid harmful effects of inbreeding, it is useful to have several

independent selected lines which can be then intercrossed with one another. And finally he discovered that "in accordance with the requirements of Michurinist genetics" selection should be carried on animals which are well fed and well taken care of, since otherwise "any breed degenerates." Using these Michurinist discoveries, M. F. Ivanov obtained a breed of swine which equals in productivity the English White (one of its progenitors) but has the sturdiness of the local Ukrainian breed (its other progenitor). He also obtained the Askania breed of sheep by hybridization and selection involving three other breeds, including a local breed adapted to climatic conditions of southern Russia. To this must be added that one of the principal works of M. F. Ivanov was published in 1916, when Lysenko, the greatest exponent of Michurinism, was still far removed from the beginning of his career.

V. A. Balmont, a laureate of the Stalin prize, made use of the "teaching" of Michurin, according tc which a breed adapted to withstand severe climatic and nutritional conditions should be selected among animals exposed to such conditions. He did obtain a new breed of sheep combining some of the sturdiness and hardihood of the local Kazakhstan breed and some of the fine wool quality of the Precox breed. The interesting work of N. S. Butarin used the "Michurinist methods of interspecific hybridization" of the Precox breed of sheep with the wild species, *Ovis arkar.* Here artificial insemination with the sperm of the wild sheep was used, and a new breed was established in 1950. The mean weight of the males is said to be 103 Kg and the females 64 Kg.

S. I. Steiman is said to have used "the materialist theory and the tenets of the creative Darwinism and the doctrine of I. V. Michurin," which led him to conclude that "the task of the breeding work is not in keeping the heredity of the animals on the level on which it is found, but in constant development and improvement. Every generation should be superior in its quality to the preceding one. This is the basis of the progress of the selection work." It is doubtless due to such novel Michurinist theories and methods that Russian animal breeders have established since 1917 a total of 7 new breeds of cattle, 5 breeds of swine, 13 of sheep, and 4 of horses. Many other new breeds are in the process of testing. This is an impressive record, which, according to Kushner, is "without precedent in the history of animal breeding."

The effectiveness of selection and hybridization as methods of animal and plant breeding should not, one may think, diminish because the invention of these methods is ascribed to Michurin and to Lysenko. After all, not even Professor Kushner claims that new breeds of swine or of sheep have been obtained by vegetative hybridization, by fertilization with mixtures of sperm of several species, and such-like Michurinist miracles. The matter has, however, a serious as well as a ridiculous aspect. Selection and hybridization were invented, of course, centuries before either genetics or Michurin were born. But it is only thanks to the development of the genetic theory that an understanding of the nature of these methods

has been and is being advanced. Genetics is just as necessary for the advancement of breeding as physiology and biochemistry for the advancement of therapeutics. The use of drugs goes back perhaps even farther than the use of selection, but replacement of medicine by witchcraft would hardly improve public health. In the long run, lying will not prove to be a good method of animal or plant breeding. It is hard to tell whether Professor Kushner believes what he wrote in his article—it seems on the whole more probable that he does not—but doubtless there are breeders who have been led to believe in all earnestness that they can learn the science of breeding from Michurin and Lysenko. Their influence on the breeding work is bound to be like that of witch doctors on the health of their patients.

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## TEMPERATURE TOLERANCE IN TETRAHYMENA

With great interest evident in biochemical and physiological studies on Tetrahymena, it has become increasingly necessary to define closely the environmental limits within which this species can survive. Some data are available in the literature concerning the temperatures employed during studies on certain strains of Tetrahymena, but little information is published on heat tolerance in the various strains. The work reported here was done to define temperature tolerance in one strain of this species.

### MATERIALS AND METHODS

Stationary phase cultures of *Tetrahymena pyriformis* strain E. were concentrated by centrifugation and resuspended in fresh culture medium used for the maintenance of stocks (Slater, 1952).[1] The concentrates used were adjusted to contain approximately 200,000 ciliates per ml. One-half ml. amounts of the suspensions were then placed in thermal death rate tubes of the dimensions 8 × 124 mm with a wall thickness of one mm. At least four tubes were employed for each temperature tested, and all of the experiments were repeated at least once. Sterile technique was employed throughout the experimentation. The pH of the suspending medium was adjusted to 6.5 by means of 0.1N NaOH.

Suspensions were first kept at room temperature for a few minutes and then placed very rapidly into a wire suspension basket in a thermostatically regulated water bath. Simultaneously with this action, a thermometer which had been previously placed in a thermal death rate tube containing one-half ml. of stock medium was transferred with the tube into the water bath. When this thermometer indicated the temperature of the water bath, timing was begun. After a given exposure to each temperature, tubes were removed quickly from the apparatus and placed in a beaker of water at room temperature.

The thermoregulator used was an immersion, flange head type with a sensitivity of 0.1° F., and the heat was supplied from a knife type immersion heater. The organisms were observed microscopically immediately after each experiment and finally 24 hours later. The thermal death time at any given temperature was defined as that length of exposure after which none of the ciliates survived more than 24 hours. Further, no motion of any kind was observed at this time and in most cases, the organisms had disintegrated.

### RESULTS

Generally speaking, large blisters were formed on many of the protozoans at one degree under any temperature which proved to be lethal for the entire population. In some instances, the blisters formed were as large as the entire organism. These extrusions of the cytoplasm were always of a clear vesicular nature.

Tetrahymena was found to be capable of withstanding temperatures as high as 44°C. for 30 seconds, but lived for only a short time after being exposed to this temperature. Immediately after being exposed to this extreme in temperature, large vacuoles were formed within the organisms. None of the animals exhibited more than slight ciliary motion however, and within 24 hours all had died. When other suspensions were subjected to 43°C., somewhat more ciliary motion was observed immediately after removal to room temperature, but here again this temperature proved lethal within 24 hours. At 42°C. a few of the ciliates were quite active after 30 seconds exposure, but many of these exhibited a folded, crenated appearance and, once more, all died within 24 hours. Exposure to 41°C. for this short exposure time had no visible effect.

Exposure to 41°C. for one minute, aside from causing the formation of small blisters, had no further visible effect, and most of the animals recovered after 24 hours. When the organisms were exposed for 2½ minutes to 41°C. this proved lethal. At 40°C. and 2½ minutes exposure, motion was considerably reduced, but all of the organisms recovered later. Further temperature effects are indicated in fig. 1. It is noteworthy that nine hours

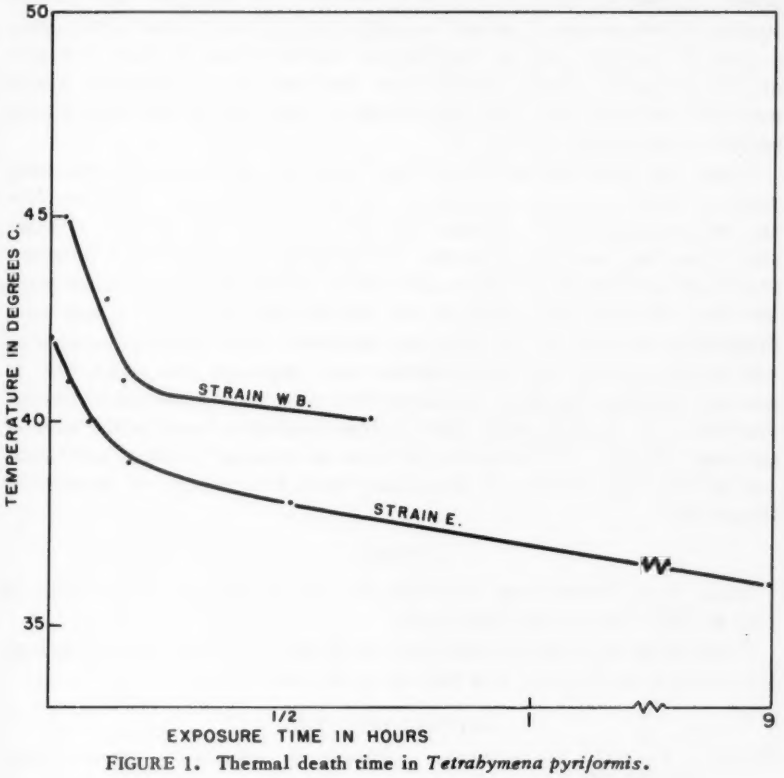


FIGURE 1. Thermal death time in *Tetrahymena pyriformis*.

exposure to 35°C. had no effect on survival in strain E. Our experience in this laboratory has indicated that daily room temperatures as low as 32°C. for several days prove to be lethal for this strain. Data from Loefer and Mefferd[2] are included on the graph for comparative purposes.

## DISCUSSION

It is interesting to note that strain E of *Tetrahymena pyriformis* is capable of withstanding temperatures as high as 35°C. for periods as long as nine hours. This is particularly true in view of the fact that optimal growth (120,000–200,000 cells/ml) for this organism is attained at about 25°C. when grown in peptone medium. Work by Elliott, Hogg, Slater and Wu[3] has shown that a double maximum for growth occurs when this strain is grown in synthetic medium, with temperature maxima at 24 and 28°C; i.e, maximum growth can be obtained at either of these temperatures.

Data from Loefer and Mefferd[2] on strain WB show that this ciliate has a lower heat sensitivity than strain E; in fact, Loefer's strain is able to tolerate 39°C. four times longer than can strain E. This is but another example of the differences which exist between the various strains of this genus. A study of this kind applied to others of the twenty-six pure culture strains of Tetrahymena (Corliss)[4] would probably reveal further differences. A great deal of the work on Tetrahymena has been done on various strains and results such as those reported here show that strict comparison of data with that obtained from other organisms is valid only if the strains concerned are indicated.

Strain HS, first isolated by Phelps[5] from an artesian well containing water at 39.5°C., is an example of a ciliate of the genus which can live at a temperature which is lethal for the two indicated strains. Further studies on the possible influence of physiological age on heat tolerance need to be undertaken. Loefer's data are presented here on the same graph with that obtained from strain E, but caution must be taken in any strict comparison because of the fact that stationary phase cultures were used with strain E while log phase cultures were employed with strain WB. In general, however, log phase protozoa tend to be more sensitive to damage than those in the stationary phase. This is true at least in the case of radiation effects on *Paramecium caudatum* as reported by Giese and Heath[6] and in the case of thermal death rate with Paramecium as reported by Doudoroff.[7]

## SUMMARY

Strain E of *Tetrahymena pyriformis* is able to tolerate temperatures as high as 39°C. for at least nine hours.

Comparative data from observations with strains E, WB and HS indicate that strain E is the least heat tolerant of the three.

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## A THEORETICAL ASPECT OF THE GENETICS OF VOLVOX

Descriptions in the literature of the development of *Volvox aureus* reveal a situation which has interesting genetic consequences. With the renewed interest in Chlamydomonas and other fresh-water algae which is apparent, it is profitable to examine some of these theoretically as a guide to experimental study.

The Volvocales are a rather homogeneous group in which there is an evolutionary series leading from the unicellular Chlamydomonas type to complex colonial forms. With a few possible exceptions these organisms are haploid, the zygote representing the only diploid cell in their life cycle. *Gonium pectorale* is a 16-cell colonial form in which the four products of meiosis persists for a time as a free-swimming tetrad. Each cell of the tetrad later gives rise to a typical 16-cell colony. In a nice study of this organism Schreiber[1] showed that pairs of cells of the tetrad were always of mating type. Tetrad isolation is particularly simple in this organism and it was even possible[1] to get evidence as to whether segregation occurred at the first or the second meiotic division. Pandorina and Eudorina are colonies having commonly 16 and 32 cells respectively. The germinating zygote produces four cells by the two meiotic divisions as in Gonium, but here three normally perish without further division and the new colony is formed by the single surviving cell.

*Volvox aureus* is one of the largest and most complex of the series, and its genetic system would appear to be unique. It is well known to be typically dioecious and to have a rather strict alternation of sexual and asexual reproduction; in either case the daughter colonies arise from a single cell.[2] Sexual fusion results in a resistant zygote in which the first two divisions are meiotic. The four resulting haploid cells, unlike Pandorina and Eudorina, remain together and by further division give rise to a single new colony.[3] This means that a Volvox individual can be made up of cells of four different genotypes, surely an unusual opportunity for students of physiological genetics. Gametes and asexually produced daughter colonies will be of any of these four genotypes at random. There is no vegetative cell division in an individual so that favorable genotypes do not reproduce preferentially.

As a consequence, Volvox has many of the evolutionary advantages of a diploid organism. Unfavorable genes or gene combinations can be carried over several sexual and asexual generations without being nakedly exposed to selection. The results of mendelian segregation will also resemble those of a diploid or tetraploid rather than a haploid organism. When two pure line diploid individuals differing in n genes are crossed, the first generation individuals are of only one genotype, and the second and following generation individuals can be of $3^n$ different genotypes. Two haploid individuals differing in n genes can give rise to $2^n$ different genotypes in the first and following generations.

Volvox will give rise to daughter Volvoces of a number of different genotypes in the first generation and to a number of genotypes intermediate between those of a diploid and tetraploid organism in the second and subsequent generations. The exact number can easily be computed; it will be the number of kinds of tetrad which can be produced from a zygote heterozygous at n loci plus some more types due to zygotes which are homozygous at some of the loci. The computation of the number of kinds of tetrad possible from zygotes heterozygous at n loci is available.[3] For the additional classes let us demonstrate the procedure by working out the case of n = 4. There will then be additional second generation zygotes with 4, 3, 2 and 1 loci homozygous. The number of ways of picking these loci will be $\frac{r!}{r!\,(n-r)!}$ where r is the number of loci homozygous. As each locus may be homozygous for either of two alleles, these will each generate $2^r$ different combinations. The totals are shown in table 1.

TABLE 1

NUMBER OF KINDS OF VOLVOX WITH ALTERNATIVES AT N LOCI

| | No. of kinds generated by heterozygous loci (3) | No. of ways of picking homozygous loci $\frac{n!}{r!\,(n-r)!}$ | No. of kinds of tetrad generated by homozygous loci $2^r$ | Total kinds of tetrad |
|---|---|---|---|---|
| 4 loci heterozygous | 48 | .... | .... | 48 |
| 3 loci heterozygous (1 locus homozygous) | 11 | 4 | 2 | 88 |
| 2 loci heterozygous (2 loci homozygous) | 3 | 6 | 4 | 72 |
| 1 locus heterozygous (3 loci homozygous) | 1 | 4 | 8 | 32 |
| 4 loci homozygous | .... | 1 | 16 | 16 |
| | | | | 256 |

In the first generation of a cross between two pure lines of Volvox, the number of different genotypes will be the number of different unordered tetrads possible.[3] In table 2 these numbers and the total possible genotypes which appear in the $f_2$ and subsequent generations are shown, for 1, 2, 3, and 4 gene differences in the parents, in the case of haploid, diploid and tetraploid organisms, and for Volvox.

It is hard to estimate the corresponding classes of phenotypes because we are ignorant of the phenotypic interactions in a living tetrad which is a coenobium.

A dominance effect for such characters as flagellum paralysis might well be communicated over the whole coenobium by means of the cytoplasmic strands connecting the cells. A factor controlling such a character is known to be transmitted across the cytoplasmic bridge of mating Chlamydomonas.[4]

TABLE 2

MAXIMUM NUMBER OF DIFFERENT GENOTYPES

| No. of loci involved | 1 | | 2 | | 3 | | 4 | |
|---|---|---|---|---|---|---|---|---|
| Generations | 1st | 2nd and following | 1st | 2nd and following | 1st | 2nd and following | 1st | 2nd and following |
| Haploid $(1+1)^n$ | 2 | 2 | 4 | 4 | 8 | 8 | 16 | 16 |
| Diploid $(2+1)^n$ | 1 | 3 | 1 | 9 | 1 | 27 | 1 | 81 |
| Volvox | 1 | 3 | 3 | 11 | 11 | 49 | 48 | 256 |
| Tetraploid $(4+1)^n$ | 1 | 5 | 1 | 25 | 1 | 125 | 1 | 625 |

The variety possible in the first generation which increases in the second generation should give Volvox quite an evolutionary boost over its algal competitors. The fact is that Volvox is more often regarded as a dead end among Chlorophyceae. Perhaps this is related to another peculiarity of Volvox, namely, the small number of offspring which an individual gives rise to, both sexually and asexually. A Volvox colony comprising 10,000 or more cells only gives rise to a maximum of about 40 or 50 daughter colonies before death.

If the observations of Zimmerman[2] and previous workers quoted by him are confirmed, *Volvox aureus* will provide an organism well worthy of genetic attention.

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# CONCERNING THE HEALING OF CHROMOSOME ENDS PRODUCED BY BREAKAGE IN *DROSOPHILA MELANOGASTER*

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# CONCERNING THE HEALING OF CHROMOSOME ENDS PRODUCED BY BREAKAGE IN *DROSOPHILA MELANOGASTER**

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## THE DEVELOPMENT OF THE CASE FOR THE RELATIVE PERMANENCE OF GENE POLARITIES IN DROSOPHILA

Analyses of numerous and varied cases of structural change of chromosomes induced by irradiation of Drosophila spermatozoa, as well as of some spontaneous cases, suggested the view (Muller, 1932, 1938, 1940a,b) that, in this material at least, broken ends of chromosomes, exposing the adhesive faces of interstitial, i.e. bipolar, genes, do not automatically become free ends, i.e. monopolar, and that, *vice versa*, free ends do not join on to each other or to broken pieces and thus become interstitial ("chromosome fusion")—processes both of which had previously been taken for granted by virtually all students of chromosome behavior and evolution, —nor do pieces join on to the side of a chromosome thread, so as to give a tripolar gene (Kossikov and Muller, 1937). Instead, it appeared that structural changes, ordinarily at least, involve unions of broken ends in a new arrangement (for diagrams of these arrangements see Muller, 1940a). Thus structural changes would constitute, in effect, "illegitimate crossovers" (Muller, 1932; Darlington, 1932, independently—a term now to be avoided, however, since it may be taken as implying the "contact first" rather than the "breakage first" mechanism of structural change) or, to use the equivalent but less committal expression of Blakeslee's, "segmental interchanges," such as the translocation "Nubbin" and the "secondary trisomics" found some years earlier in Datura by Blakeslee and his co-workers. It was therefore considered appropriate to distinguish any monopolar chromosome end by a special term, "telomere."

That this distinction was not of absolutely universal validity (i.e. that changes can occur in polarity) was evident from the beginning on evolutionary considerations, and was illustrated by the normal fragmenting of chromosomes in the life cycle of Ascaris (Boveri, 1904) and their temporary fusion in that of *Lymantria monacha* (Seiler and Haniel, 1921), as well as by the findings of Stadler (1939, 1941), McClintock (1939, 1941) and others, showing the automatic, seemingly adaptive conversion reaction of ends broken by radiation or mechanically into free ends in maize sporophyte (but apparently not gametophyte or endosperm) cells.

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It was also thought that exceptional cases, illustrating the origination of free from broken ends, and of interstitial regions from free ends, existed in Drosophila. Chief among the older established cases of these were the following (the order here used is far from chronological): (1) apparent "detachments" of attached-X chromosomes; (2) one or more supposed cases of terminal inversion (spontaneous) that had been found and studied genetically by Sturtevant (especially In3RC, see Sturtevant, 1931); (3) a supposed terminal deletion of the X known as scute-19 (Muller, 1932); (4) a case (scute J4) of attachment of a subterminal piece of the X to what appeared to be the left end of chromosome III; and (5) numerous, frequently occurring cases (first found by Levit, 1931, see Muller, 1932) of apparent loss of the left tip of the X, including the loci of $y^+$ and $ac^+$, after (and even without) irradiation of the scute-8 chromosome. In this X chromosome heterochromatin has been transferred by a long inversion from the region near the centromere to a much more distal position, just proximal to the $y^+$-$ac^+$-containing region subject to loss; obviously, then, this transferred heterochromatin, like heterochromatin in its original position, is readily broken, and—judging by appearances—its breaks heal readily. (6) In addition, supposedly branched chromosomes, illustrating tripolarity, were thought by Bridges to be present in the case of the Pale translocation (which also supposedly illustrated a terminal deletion of chromosome II), and (7) in that of Dominant-eyeless (Bridges, 1935). Moreover (8), various cases of apparent "fusion" of the right end of the X chromosome with the fourth chromosome, and of pieces of other chromosomes with the ends of X and IV, were described cytologically by the University of Texas group of Drosophila workers in the middle '30's (see for instance Painter and Stone, 1935), and these and the origination of (9) the ring X-chromosomes, $X^{c1}$ and $X^{c2}$, and (10) attached X-chromosomes appeared to give further illustrations of the conversion of ends to interstitial regions.

One by one, however, all these cases fell through or were thrown into such serious question as to make them inadmissible as evidence of change in gene polarity.

(1) Apparent detachments of attached-X's were shown by Philip (1935) usually to result from crossing over (or at least some sort of segmental interchange) with a Y.

(2) The supposed terminal inversions were found on salivary analysis by Bridges to have a second break, in one case extremely close to the chromosome end (Morgan, Bridges and Schultz, 1937).

(3) The scute-19 deletion also was found, both genetically (Muller, 1935) and on salivary analysis (Muller, Prokofyeva and Raffel, 1935), to be a minute interstitial instead of terminal deletion, for despite the fact that there was a break just proximal to the scute locus and therefore very near the end there was another still nearer the end, and the end region itself was present.

(4) In the scute J4 case the stump of the X had conjugative properties indicative of its having received, reciprocally, a most minute terminal

region from chromosome III. More telling, however, was the consideration that the idea of change of polarities would in this case have required the simultaneous and independent change of the originally free end of chromosome III to an interstitial region and, contrariwise, of a broken end, representing an interstitial region, of the X chromosome to a free end, whereas the other view involved only the assumption that one of the breaks was exceedingly close to the end of chromosome III.

(5) Genetic analysis showed that some 20 per cent of the apparently "terminal" deletions of the scute-8 chromosome still contained *lJ1*$^+$, the normal allele of lethal J1, which was only slightly distal to ("left of") the loci of yellow$^+$ and achaete$^+$ that had been "lost"; while a few even had the normal allele of the lethal which lies nearest $y^+$ on its left side, and were therefore viable *y ac* "mutants." These 20 per cent therefore were results of double breakage, and were probably mostly deletions, although some may have been minute inversions with position effects. It was only to be expected, both on considerations of map length and of the presumably higher breakability of the small terminal heterochromatic region at the left end of the X (see below) that most of the deletions would not fall short of *lJ1*$^+$, even though they were interstitial, but in their case cytological evidence of the presence of some of the terminal heterochromatin was provided by observations of the structure and conjugating properties of the salivary chromosomes (Muller, Prokofyeva-Belgovskaya and Raffel, 1937; Raffel, 1938).

(6) The Pale translocation was shown by salivary examination (Kossikov and Muller, 1935, and independently Morgan, Bridges and Schultz, 1935) to have involved an interstitial instead of terminal deletion (the more distal break being very near the end) of chromosome II, with insertion, instead of side-attachment, of the deleted fragment, in linear order, into the space left by a break in chromosome III, all polarities being retained.

(7) Dominant-eyeless similarly received its explanation as a deletion-insertion (now also called "transposition," when from one chromosome to another), but one in which the inserted piece had consisted of two identical (sister) chromatid fragments which, though joined linearly at their breakage points in mirror image arrangement, protruded sideways from their region of insertion because of their conjugational attraction for one another (interpretation of Muller; see Offermann, 1936).

(8) The cases of apparent fusion with the right end of the X, or with the supposed centromere-bearing end of chromosome IV were explained by the findings that the X-chromosome has a tiny arm (later shown to be entirely heterochromatic) to "the right" of its centromere (Prokofyeva, 1934), and that the fourth chromosome likewise has its centromere subterminally placed, with one arm (which, however, is termed the "left") minute and entirely heterochromatic (Panshin and Khovostova, 1938; Griffen and Stone, 1939); breaks in these arms, followed by attachment of a different chromosome-piece to the proximal "stump," would therefore give the appearance of terminal attachments.

(9) The ring X-chromosomes were proved on salivary examination (Schultz and Catcheside, 1937) to be real cases of terminal deletion, of both the distal left and right heterochromatic regions, in which, however, instead of healing, the breakage points (or, more properly speaking, "faces") had joined to one another so as to form the ring, and not to be cases of distal ends becoming attached.

(10) It became probable that the attached X-chromosomes had arisen by the reverse of the process whereby they usually became detached: namely, that an X which by one crossing over with a Y had acquired a Y-arm then, by crossing over between this arm and another X, acquired a second X-arm in place of the Y-arm, and some cases of this process of origination of attached X's were found; however, it is to be expected that attached X's would, more rarely, arise directly, by double breakage and exchange between a part of the right arm of one X and the left proximal heterochromatic region of another, and also, indirectly, by a process in which the fourth chromosome took the place of the Y as intermediator.

That any cases at all should have been found in which the breaks were so extremely close to the end of the chromosome as was proved for several of the above cases, and made probable for others, and which thereby simulated changes in gene polarity, became understandable in the light of two series of findings. One of these was the evidence showing the unusually high tendency of heterochromatic regions, and probably of regions near them, though to a lesser extent, to undergo breakage and rearrangement (Muller and Gershenson, 1935; Muller, Prokofyeva-Belgovskaya and Raffel, 1937; Belgovsky and Muller, 1937; Belgovsky, 1938; Muller, 1938, 1941, 1944). The other finding was the cytological one that heterochromatin exists not only in the proximal (centromeric) regions of chromosomes, but that there is also a minute heterochromatic region at each distal end of each chromosome, as shown not only by its structure but by its proclivity for conjugating with other heterochromatin (Prokofyeva-Belgovskaya, 1937, 1938). It thus became probable that breaks were especially likely to occur not only near the centromere but very near to any distal end, a conclusion which fitted in with certain earlier observations on the distribution of breaks in cases of translocation (Patterson, Stone, Bedichek and Suche, 1934).

Besides the cytogenetic analyses of the series of scute-8 cases certain other experiments were carried out which had as one of their objects the testing of the possibility of simple breakage with healing. Thus, in 1931 and 1932 two large scale searches were conducted for scute mutants, by Dr. Jessie Jacobs-Muller and by Miss Hennie Levy, respectively, under the direction of the senior author (these are largely unpublished, but see Muller, 1932), among the daughters of scute females crossed to heavily irradiated normal males. Some 14 scute stocks were thereby obtained (designated as $sc^{J1}$ to $sc^{J6}$ and $sc^{L1}$ to $sc^{L8}$, the notation in Bridges and Brehme's (1944) "The Mutants of *Drosophila melanogaster*," to the effect that the letter L in these symbols denotes Levit's name being incorrect).

Despite the finding, in later analyses, that many of these mutants involved breaks in the euchromatin close to the right of the scute locus, none proved to represent a single healed break, i.e. a terminal deletion resulting in loss of the scute locus. Nevertheless, special tests proved that, had such a mutant arisen, it would have had a fair chance of surviving in these experiments. From time to time other scute mutants, obtained in a similar way (i.e. heterozygously, in the female, so as not to miss those lethal to males), have been tested and have given results entirely concordant with these, there being no case in which the terminal region of the X disappeared though it was often transferred in position. It should be noted that in this work it was the euchromatin while in that on scute-8 it was the heterochromatin which was being tested for the likelihood of healing following breakage.

Another series of tests was made by Belgovsky (1938, Belgovsky and Muller, 1937), under the direction of the senior author, using for irradiation with 4–6000 r males having an analogue of the scute-8 chromosome, namely, the structurally similar Bar-M2 chromosome. This has a moderate-sized inversion, with one of its breaks in the proximal heterochromatic region and the other rather close to the right of the locus of forked$^+$ (instead of close to the loci of $y^+$ and $sc^+$ as in scute-8). In this case, if a single break with healing occurred in the especially breakable heterochromatic region just proximal to $f^+$, the resulting fly would be a viable male (as tests with the similarly sized fragment provided by the Bar-Stone translocation had shown). In fact, the $B^{M2}$ stock was chosen for this work chiefly because such flies would be viable, and because breaks on the given position would be frequent. Moreover, the markers were so arranged (the mother being *sc v f car*) that such a case would be recognizable (as a *sc v f car*$^+$ male). None, however, was found in a count of 15,831 $F_1$ males. Instead, numerous exceptions were found that fell into the following three classes: (1) large paracentric deletions with one break near the centromere and the other near the distal end but to the right of $y^+$; (2) large paracentric deletions with one break in the heterochromatic region just proximal to $f^+$ and the other near the distal end, but to the right of $y^+$; these proved that, as expected, breaks were in fact frequent in the transferred heterochromatic region near $f^+$; (3) apparent losses and other changes (some variegated in expression, as happens with $y^+$ in scute-8 chromosomes) of the locus of $f^+$, many of them occurring simultaneously with losses or changes of neighboring genes, as shown by the linked recessive lethal effects. The third group showed clearly that minute interstitial deletions and probably other rearrangements (mainly inversions) were being produced in and near the heterochromatic region just to the right of $f^+$ and in the $f^+$ region itself, yet without loss of the distal part of the X chromosome. Thus these results confirmed and made more definite the interpretation given above of the scute-8 cases (and of parallel findings being then made with the similar scute-S1 chromosome), as being minute double-break interstitial rearrangements, with one break in and the other close to or in heterochromatin,

and underlined the absence, especially in heterochromatin, of single breaks that had healed.

Several other series of findings were made thereafter which seemed to reënforce the conclusion that chromosome breakage in Drosophila seldom if ever results in change in gene polarity. Among these was the evidence (Muller, 1940b, 1941; Pontecorvo and Muller, 1940; Muller and Pontecorvo, 1941; Pontecorvo, 1941, 1942) that breakage occurs far more frequently than had been thought, that the great majority of these breaks undergo restitution, but that of the rest the great majority do not succeed in uniting with ends derived from other breaks, but act as dominant lethals, not through the hypoploidy engendered by the chromosome loss but through the effect of the breakage-fusion-bridge cycles occasioned by the union of the broken ends of sister chromatids with one another. In other words, breakages are very plentiful in irradiated material (and not very rare even in control material), yet despite all this opportunity for healing what regularly occurs instead is union between broken ends, even when the chromosomes have to wait for the duration of a cell cycle, with its chromatid reproduction, before there is opportunity for the broken ends to find nearby complements to unite with.

In an attempt to obtain evidence for or against healing in material presenting the advantage that breakage would not result in aneuploidy, Schultz (personal communication) irradiated spermatozoa containing ring X-chromosomes and tested the offspring genetically for the presence of "rods." Since a large proportion of breaks occur in the heterochromatin these, if followed by healing, would have produced "rods" capable of giving abundant crossovers with other rods of the same orientation; thus these cases would be recognized. None were found, however. Later, Lea and Catcheside (1945) carried through a larger scale experiment of essentially the same kind, and also obtained negative results. They then proceeded to point out that their test is after all, despite its scale, not a very sensitive one, since the production of a viable rod would require simultaneous healing of both breakage faces, i.e. of both of the potential ends of the rod, a requirement that would (if healing of the two ends occurred independently) make the cases of rods only about as frequent as the square of the frequency of healing of an individual break, multiplied by the frequency of the individual breaks. They thereupon, despite their results, preferred to retain their view that healing probably occurs in Drosophila.

In maize Stadler (1939, 1941) has reported a much higher frequency of terminal deletions, involving healing, in relation to cases of interchange of connections of broken ends, after ultraviolet than after doses of ionizing radiation that give comparable total breakage rates. It is therefore of especial interest that McQuate (1950, 1954), working under the senior author's direction, found no cases giving evidence of breakage of the Y chromosome with healing after irradiation of Drosophila spermatozoa with ultraviolet, although a considerable number of losses of the Y best

ascribable to the breakage-fusion-bridge phenomenon were found, and three cases of double breakage with interchange of connections involving the X, and the genetic set-up was so arranged that a high proportion of cases of single breakage of the Y with healing would have been recognized.

## CONTRARY EVIDENCE

During the later years of the development of the original case in favor of the relative permanence of gene polarities, namely, in 1936–1941, several new series of observations and experiments on Drosophila were published which again appeared to call for the interpretation that changes in gene polarity had occurred. Earliest of these was a related series of studies by workers of the Cold Spring Harbor group and by Kikkawa. Kaufmann (1936) first reported an apparently terminal inversion seen in salivary gland preparations of *D. ananassae*, and what was evidently a representative of this same inversion was reported again by Kikkawa (1937). As with Sturtevant's Inversion 3RC, such a case would involve the simultaneous change of the end (monopolar) gene into an interstitial (bipolar) one, and of the interstitial gene on the distal side of the single breakage point into an end gene, i.e. healing. It is not clear, however, whether a breakage point within the minute subterminal heterochromatic region, so extremely close to the end as found in the case of *In3RC*, would have been recognized in these studies. A similar stricture applies in the case of several salivary chromosome figures suggesting terminal deficiencies in *D. melanogaster* presented by Demerec and Hoover (1936) and in *D. ananassae* by Kikkawa (1938), since at that time the very existence of terminal heterochromatin, which is especially elusive and variable in its fixation and staining, as well as of genetically variable actual size, had not yet been generally realized.

Sutton (1940a,b) reported five deficiencies of the left end of the X chromosome of *D. melanogaster* which appeared terminal in salivary preparations. One of these seemed to involve the interstitial insertion of the supposedly terminal fragment, a case, like that of terminal inversion, which would have to involve two simultaneous changes of polarity, in opposite directions. It is relevant to note in this connection that in another salivary chromosome study, dealing with details of the yellow-scute region (a region included within that of the deficiency studies just mentioned), the observations of Sutton (1943) on the location of the breaks affecting these loci are in irreconcilable conflict (although she did not note this fact), with both the published and unpublished findings of Prokofyeva, which were most extensive, meticulous, and consistent with one another, and which had been carefully verified by the present senior author as well as by Raffel and others (see for instance Muller and Prokofyeva, 1935; Muller, Prokofyeva and Raffel, 1935). In any case, no matter how likely or unlikely it may seem that some individual instance represents a true terminal deficiency or inversion, proof that this particular aberration is really terminal can not be obtained, since there is no way of excluding as an ex-

planation in any given instance the occurrence of an interstitial rearrangement in which the telomere section remaining is minute enough to escape cytological detection. Therefore only quantitative studies, such as for example one in which the frequency of *apparently* terminal deficiencies found is greater than that expected for non-terminal deficiencies in which the terminal section was invisibly small, can demonstrate that there is a class of mutations in which genes change their polarities (Muller, 1941).

Finally, Bishop, also while working at Cold Spring Harbor, in an unpublished genetic study (discussed by Fano, 1941) of the composition of a series of X-chromosomes having large induced deletions, found several cases in which the left-most marker used was not present, as though the deletions had been terminal ones produced by single breakage in the proximal region, followed by healing, or else had involved a break so distal as to be to the left of yellow. Fano concluded from the results that the frequency of these cases was too high to have been ascribable to interstitial deficiencies, and, that possibly as many as 5 per cent of the breaks which occurred underwent healing. It may however be recalled that in the experiments of others a not inconsiderable number of two-break cases has been found (in view of the very small length of chromatin in question) in which one of the breaks was definitely proved to be to the left of yellow. The best known of these are $y^{3P}$, $sc^{J1}$, $sc^{19}$, Stone and Griffen's "free centromere" deletion (1937), $X^{c1}$ and $X^{c2}$, and the numerous minute deletions that have been produced (with the other break in the transferred heterochromatin close to the right of the locus of $y^{+}$) in scute-8 and scute-S1 chromosomes.

The most convincing of the evidence for the healing of broken ends is that obtained in a brilliantly conceived experiment reported by Rapoport (1940, 1941). Owing to the fact that these papers were published only in the U.S.S.R., during the war, we did not become aware of the significance of their contents until 1952, and it was in fact the challenge of the results therein reported that then decided us to make a reinvestigation of the questions involved.

In the main work reported by Rapoport, healing was investigated by looking for cases of detachment of attached X's among the offspring of irradiated females that had contained attached X's but no Y chromosome. The absence of a Y (it was not explained how this was achieved) insured that detachment could not occur by the usual method of "crossing over" between X and Y. Since detachment was still possible through the occurrence of a large interstitial deletion in one of the X arms, markers were so arranged that such a deletion would be recognized if its distal break had occurred (as it would usually be expected to occur) anywhere to the right of the *y ac* group of loci. At any rate, there should be many more deletions recognizable as such in this way than those not recognizable. It was therefore natural to infer that if there were many more cases of detachment which did not give genetic evidence of being deletions than of those that did, most of the former would have resulted from single breakage followed by healing.

There are several advantages evident in this approach. First, all previous experiments on the subject had involved irradiation of spermatozoa, and just as in maize healing had under some circumstances been found to occur in sporophyte but not in gametophyte or endosperm cells, so it might be that the peculiarities of spermatozoan chromosomes in some way militate against the healing process. Second, it has long been known that in other cells than spermatozoa gross structural changes involving two or more breaks, with reciprocal interchange of connections, occurs far less frequently, for a given dose of radiation, than when spermatozoa are irradiated. This would lessen the complication caused by the presence of such cases along with those resulting from single breakage and healing, provided the latter did occur. Third, unlike what is true of ring chromosomes, healing of only one of the two "breakage-faces" formed by the single breakage is necessary to give a recoverable exception when attached X's are used. Fourth, with attached X-chromosomes, a high proportion of the cases of single breakage with healing (supposing they occur) would result in viable, recognizable exceptions, free from a damaging amount of hyperploidy. For there would be no significant reduction of viability caused by hyperploidy if the break took place anywhere in the proximal heterochromatic region of one of the arms (and a fourth to a third of all breaks in the X do occur in this region), and only a moderate amount if the break occurred anywhere in the considerable stretch of euchromatin to the right of the locus of $f^+$. Thus the occurrence of healing in both kinds of chromatin would be tested.

One of the arms of the attached X's in Rapoport's experiment was provided with the markers *y apr fa f* (*apr*, the symbol for apricot eye, having at that time been represented by $w^a$), and the other arm with $y^2$ *Hw* and the long inversion *Cl* derived from the *ClB* chromosome, although *B* was not present (see fig. 1). The occurrence of crossovers between the arms would in effect be prevented (at least in some 99 per cent or more of the viable eggs) by the *Cl*; the loci of *y* and *Hw* have never been known to cross over with one another anyway. With attached X's of this composition, if breakage followed by healing occurred somewhere in the proximal region of the *Hw*-bearing arm, and the egg received the centric X, i.e. the entire other arm, with only the stump of the arm which had borne *Hw*, fertilization by a sperm with a Y would produce a male of phenotype y apr fa f. If on the other hand the healed break had occurred in the proximal region of the *apr*-bearing arm, the genetically $y^2$ *Hw Cl* male formed by fertilization with a Y-bearing sperm would die because of the recessive lethal associated with the inversion *Cl*. Supposing that the sperm had carried an X instead of a Y, and that (as seems probable but is not directly stated by Rapoport) this X had the normal alleles of the marker genes, the former type of exceptional egg would produce a wild-type female while the latter would produce a phenotypically Hw female. Both would be distinguishable from the unaffected attached-X-containing daughters since these, like their mothers, would be phenotypically y as well as Hw. In addition, depending upon culture conditions and genetic "background," some sterile triplo-X

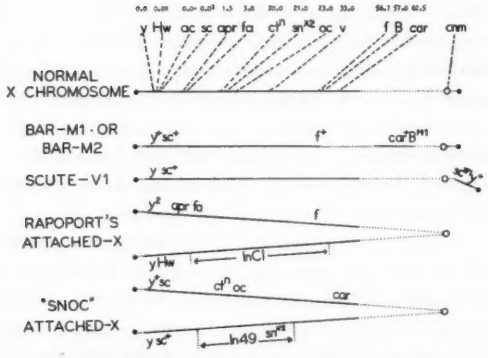


FIGURE 1. Diagrammatic figures (not to scale but with greater magnification of regions more crowded with markers) of the normal and rearranged X chromosomes here used, and the loci of some of their contained genes. Large hollow circles represent centromeres (cnm), smaller solid circles telomeres. Broken lines represent heterochromatic regions; to indicate breakage likelihood, these are shown with relative lengths similar to those which they have in mitotic chromosomes, although at the time of breakage in our experiments the heterochromatic regions were doubtless much shorter relatively, as in salivary chromosomes. (Their greater mitotic lengths result from the expansion of their blocks, however, while the blocks are not the cause of their high breakage frequency.)

"superfemales" are also to be expected, independently of the irradiation. These would resemble the Hw females resulting from breakage in being non-yellow and Hw, but would on careful inspection be likely to show the abnormalities often associated with such aneuploids, such as tendency to incision of the wings, squat body, irregular bristles and ommatidia, etc.

These attached-X females, after having been exposed to 2000 r of X-rays, gave rise to the following exceptional offspring (the number of unexceptional offspring not having been stated): 69 y apr fa f males (none showing Hw), 128 non-Hw (presumably wild-type) females, and 116 Hw (presumably non-yellow) females. There was no mention of crossovers, although several classes of them were to be expected at very low frequencies. The complete absence of Hw among the y apr fa f males was taken to mean that there were no cases of two-break deletion, with the distal break to the right of *Hw*, in the *Hw*-bearing arm (see fig. 1), and therefore in all probability no two-break deletions at all, since of course those with breaks to the left of *Hw* would at best have formed but a small minority of all viable two-break deletions.

If spermatozoa had been X-rayed a considerable number of long viable two-break deletions of the type in question, with one break to the right of the *y Hw* complex of loci and the other in the proximal region would have been obtained in any experiment using 2000 r and done on the scale on which Rapoport's experiment must have been done (see results of Muller, Koerner and Vogt, reported by Muller, 1938). In the light of this comparison, and of other early experiments (see, e.g., Muller, 1930) showing gross

rearrangements to be produced extremely rarely when cells other than spermatozoa are irradiated, the occurrence of numerous exceptional (detached-X) males in Rapoport's experiment, taken in connection with the finding that none of them was of the deletional Hw type, seemed to constitute conclusive evidence that the exceptions were produced by single breakage with healing. This conclusion seemed strengthened by several other experiments reported by Rapoport (1940, the details of which were not given), in which again attached-X females were irradiated. In these experiments (1) no evidence was obtained for translocations involving the irradiated X's; (2) crossing over of some sort was excluded as an explanation since eggs laid as early as 48, 24, and even 10 hours after irradiation, and therefore long past the stage for crossing over, frequently produced exceptions; and (3) no difference was found in the frequency of breakages no matter whether or not a Y chromosome was present in the females.

## REINVESTIGATION OF HEALING OF X CHROMOSOMES IRRADIATED IN SPERMATOZOA

So cogent did Rapoport's evidence appear to be that it was decided, in 1952, not only to conduct an investigation dealing, like his, with attached X's, but also, while waiting for the appropriate stocks of attached X's to be constructed, to reinvestigate the possibility of the healing of chromosomes irradiated in the spermatozoon stage. Two types of X chromosomes were chosen for the study involving irradiation of males. The first of these used was the Bar-M1 ($B^{M1}$) chromosome. This has a structure very similar to that of the independently arisen Bar-M2 chromosome used by Belgovsky, and therefore would be expected to have the same advantages; yet it is not identical with $B^{M2}$, as Offermann has shown in unpublished work. It was thought better to use a chromosome of independent origin from that used in Belgovsky's work in order that the case for healing might be provided with another chance to prove itself, in the event that the $B^{M1}$ stock had happened to have some special peculiarity which interfered with healing, or with the recovery of the chromosomes thereby produced.

The $B^{M1}$ chromosome (see fig. 1) contains an inversion with one break between the loci of forked and Bar and the other in the proximal heterochromatic region, so that some heterochromatin has been transferred to a position close to the right of $f^+$. This results in a comparatively high induced breakage frequency in that part of the chromosome. If single breaks there became healed, proximal fragments would be produced which contained all the euchromatin which in a normal X chromosome extends from a point close to the right of forked up to the proximal heterochromatin, as well as that heterochromatin. Included in this region would be $car^+$ (the normal allele of carnation) and the locus of Bar, which in this chromosome gives, as a result of its change in position, a "moderate-Bar" ($B^M$) phenotype.

In the present experiment irradiated $B^{M1}$ males which were otherwise phenotypically normal were crossed to females containing attached X's,

constructed for the purpose, which were homozygous for *y sc v f car* and also carried a Y. Eggs with these attached X's, if supplied with a fragment of the above mentioned type, produced by single breakage and healing, would give rise to phenotypically y sc v f car$^+$ females that were both viable and fertile (and often slightly Bar) while those eggs which were fertilized, instead, by a sperm containing a two-break deleted X chromosome having one break in the given position, close to the right of $f^+$, and the other in a distal position, would ordinarily give rise to $y^+$ sc$^+$ v f car$^+$ females, which would also be viable and fertile (and often slightly Bar). More rarely, one would expect to find two-break deletions in which the distal break was distal to (to the left of) the $sc^+$ locus; these would result in y sc v f car$^+$ females phenotypically like those which resulted from healed breaks. In addition, some double-break paracentric deletions would have their proximal break to the right of *car*$^+$. These would give either $y^+$ sc$^+$ v f car females or y sc v f car females phenotypically indistinguishable from their unexceptional sisters, depending upon whether the distal break had been right or left of the $y^+$ $sc^+$ region. Other possibilities of obtaining exceptions will be discussed along with the results of the experiment.

The technique of breeding was to use virgin $B^{M1}$ males for irradiation, and to expose them within 24 hours of their eclosion to about 4000 r of X-rays. They were then immediately mated with virgin females of the attached-X composition previously described. After six days with these females they were placed with other virgin females of the same genetic composition, and left with them until the 12th day, when the males were discarded. The $F_1$ females from the two sets of mothers were scored and recorded separately. This method of breeding was followed in order to provide additional opportunity for the demonstration of healing, in view of the recent work by Lüning (1952a,b) indicating considerable differences in the breakage and structural-change frequencies between sperm released in the first and second weeks after the irradiation of young males.

The phenotypes of the $F_1$ females found are given in table 1. There were 11 exceptional females which obviously represented two-break interstitial

TABLE 1

PHENOTYPES OF $F_1$ FEMALES FROM CROSSES BETWEEN ATTACHED-X *y sc v f car*/Y$^+$ ♀♀ AND X-RAYED BAR-M1/Y$^+$ ♂♂

| Phenotype | No. of individuals | No. in brood I | No. in brood II | Interpretation |
|---|---|---|---|---|
| y$^+$ sc$^+$ v f car$^+$ | 7 | 3 | 4 | Two-break deletion |
| y$^+$ sc v f car$^+$ | 2 | 1 | 1 | |
| y$^+$ sc$^+$ v f$^+$ car$^+$ | 1 | 0 | 1 | |
| y$^+$ sc$^+$ v f car | 1 | 1 | 0 | |
| y sc v f car$^+$ | 1 | 1 | 0 | ? |
| y sc v f$^+$ car$^+$ | 3 | 2 | 1 | |
| y sc v f car (Unexceptional) | 910 | 669 | 241 | |

deletions of the long left arm of the X, i.e. paracentric deletions, since they showed the $y^+$ of the distal portion of the left arm and of course possessed a centromere. In 9 of these 11 the right break probably occurred in the distal heterochromatic region of the $B^{M1}$ inversion since it was between the loci of $f^+$ and $car^+$, giving the f car$^+$ phenotype, while in a tenth the right break was in the euchromatin between $v^+$ and $f^+$ (giving v f$^+$ car$^+$), and in the eleventh it was to the right of $car^+$ and therefore probably in the proximal heterochromatin of the right arm. As for the left break, it was in all but two of these 11 cases to the right of $y^+$ and $sc^+$, between these loci and $v^+$, since the phenotype, in regard to these loci, was y$^+$ sc$^+$. However, surprisingly enough, in the other two cases although the left break was to the right of $y^+$ (giving the y$^+$ phenotype) it was either to the left of $sc^+$ or at least so close to that locus that the juxtaposed heterochromatin, by position effect, weakened the manifestation of the $sc^+$ gene enough to give the sc phenotype. The study of other cases (Muller and Prokofyeva, 1935; Muller, 1935; Raffel, 1938; Raffel and Muller, 1940) has shown that the region thereby defined is an extremely minute one, probably extending over only 3 loci (those containing the normal alleles *ac*, *sc*, and the lethal immediately to the right of *sc*). As the right break in both these cases was between $f^+$ and $car^+$ (being evidently in the distal heterochromatin of the $B^{M1}$ inversion), the phenotypes were y$^+$ sc v f car$^+$.

Finally, there were 4 females which had the marker $car^+$ (one of these also with $f^+$) but which manifested both y and sc. In these cases therefore the hypothesis of "terminal deletion," i.e. of a single break with healing of the broken end, remains a possible one. Other possibilities, however, are the following. (a) There was another break, very distally located, i.e. to the left of $y^+$, with the resultant formation of a paracentric deletion lacking the lefthand markers; such breaks would certainly be expected much oftener than those giving a y$^+$ sc phenotype. (b) The other break was in the small heterochromatic region to the right of the centromere of the X, so that the fusion of breakage points resulted in a ring-shaped terminally deleted X that lacked both left and right ends of the chromosome. Since it is known that breaks occur with unusually high frequency in heterochromatic regions, even when these regions are distal ones, these 4 cases do not seem an excessive number for types (a) and (b) taken together, in view of the eleven two-break deletions with a distal break to the right of $y^+$. As will be noted subsequently, however, there are still other ways, involving translocation, whereby exceptions resembling the results of single breakage with healing could be produced by structural changes in which broken ends unite exclusively with one another and do not change into free ends. Unfortunately none of the 4 females in question was sufficiently fertile to allow the establishment of a stock for the purpose of cytological or genetic analysis.

Although the above results, for the reasons given, cannot be regarded as affording evidence for healing, they do not actually disprove the pos-

sibility of it either, but only indicate that if it occurs its frequency, relatively to the number of individual breaks which are undoubtedly taking place in an appropriate position, must be very low. On the other hand, the results do raise the question why in Belgovsky's experiments with $B^{M2}$ no cases of the type resembling healing were found. Perhaps one of the reasons was the fact that the females to which his irradiated males were bred had separated X's, so that his exceptions were necessarily males. For aneuploidy of the section of the X in question, between $f^+$ and the centromere, is known to reduce the viability of a male much more than that of a female, and since together with this there would, in the cases simulating healing, be the abnormality caused by the individual being scute instead of nonscute, there might be a synergism of damage such as to allow few of these exceptions to survive.

The second series of experiments involving the irradiation of males was carried out in the same way as described for the first series, so far as the age of the males, dose of irradiation, passage through two periods of 6 days each with different lots of females, and genotype of females used were concerned. However, a different type of X chromosome was used in the irradiated males, especially designed for the detection of the presence or absence of very nearly the extreme right as well as left ends of the chromosome when a breakage product of it was found. This allowed the obtaining of evidence regarding the possible healing of breaks in the proximal heterochromatic region to the left of the centromere, much like the evidence regarding breakage in the transferred heterochromatin close to the right of $f^+$ in the preceding experiment.

The X chromosome in these males (see fig. 1) had its long left arm of normal structure, but the gene for yellow, *y*, was present near its distal end, along with the normal allele of scute, $sc^+$. The tiny right arm of the chromosome had its distal portion replaced by a segment originally derived, by a pericentric inversion, from the left end of a normal X chromosome. This segment extended from the terminus up to and including $y^+$, $ac^+$, $sc^+$ and the $l^+$ locus just proximal to that of scute$^+$. However, by position effect, the gene which had been $sc^+$ now functioned as an extreme *sc* allele, known as $sc^{V1}$. The phenotype of the males was nevertheless normal, since the $y^+$ in the duplication to the right of the centromere "covered" the *y* in the left distal region and the $sc^+$ in that region covered the $sc^{V1}$ in the right end region. This chromosome had been derived by crossing over between a *y*-bearing chromosome of normal structure and a scute-V1 chromosome having both left and right parts of the original pericentric inversion. Its symbolic designation is $y \cdot Dp\ sc^{V1}$. This symbology is misleading, however, in considering the roles of the markers, since actually the normal allele $sc^+$ serves to mark a point near the left end and $y^+$ a point near the right end, so that a more heuristic symbol would be $sc^+ \cdot y^+$.

If a breakage occurred in or near the proximal heterochromatin of the left arm, and were followed by healing, one would obtain females which were phenotypically $y^+$ sc v f car, or, if the break were between $f^+$ and $car^+$,

they would be $y^+$ sc v f $car^+$. Most surviving two-break deletions, on the other hand, would have a distal break in the left arm to the right of $sc^+$, and would therefore appear $y^+$ $sc^+$ v f car, or, $y^+$ $sc^+$ v f $car^+$. However, the relatively few two-break deletions whose distal break was left of *sc* would appear $y^+$ sc v f car, or, $y^+$ sc v f $car^+$, like results of healed breaks, and these phenotypes would also be given by the very rare cases of viable ring chromosomes resulting from pericentric deletions with one break in the right arm distal to $y^+$. Other possibilities remain to be mentioned.

The phenotypes of the $F_1$ females obtained from these studies are given in table 2. Five exceptional females, of phenotype $y^+$ $sc^+$ v f car, were

TABLE 2

PHENOTYPES OF $F_1$ FEMALES FROM CROSSES BETWEEN ATTACHED-X *y sc v / car*/$Y^+$ ♀♀ AND X-RAYED y·$sc^{v1}$/$Y^+$ ♂♂

| Phenotype | No. of individuals | No. in brood I | No. in brood II | Interpretation |
|---|---|---|---|---|
| $y^+$ $sc^+$ v f car | 5 | 4 | 1 | Two break paracentric deletion |
| y $sc^+$ v f car | 1 | 0 | 1 | * |
| $y^+$ sc v f car | 1 | 1 | 0 | ** |
| y sc v f $car^+$ | 1 | 1 | 0 | *** |
| y sc v f car (Unexceptional) | 1221 | 806 | 415 | |

*Distal parts (at least) of both arms lost, presumably followed by ring formation of remainder, but conceivably by its insertion into autosome.

**Only tip of left arm of X survived, presumably by translocation to autosome.

***Tip of right arm survived by translocation to autosome, *or* single breakage in proximal part of left arm, followed by healing.

clearly two-break paracentric deletions, since the markers for both ends were present. They had had one break between $car^+$ and the centromere, probably in the proximal heterochromatin, and the other in a left distal position to the right of $sc^+$.

A sixth female, of phenotype y $sc^+$ v f car, had the subterminal portion near the left distal end represented by $sc^+$, but since the region of the left arm to the right of this was missing, and also some at least of the small right arm (that represented by $y^+$), it seems most likely that the centromere of the X was absent and that the left subterminal fragment of the X had become attached by translocation to an autosome, at a point so near a distal end (telomere) of the latter as not to cause death of the individual heterozygous for the resulting minute deficiency of the subterminal autosomal piece for which the $sc^+$-bearing end of the X had been substituted. It may be presumed that the rest of the X, failing to become attached reciprocally, to the subterminal autosomal piece, was lost by the usual breakage-fusion-bridge cycle.

Two other exceptional females also showed a marker of only one arm. One of these, being $y^+$ sc v f car, can be explained by the same mechanism as that outlined above, if only we suppose that the subterminal portion of

the small right arm of the X, bearing $y^+$, instead of the end of the left arm, had become substituted for an autosomal end. Nevertheless, this case is, alternatively, capable of interpretation as a result of a single break in the proximal portion of the left arm, between $car^+$ and the centromere, followed by healing of the broken end. Unfortunately, both this and the preceding exceptional female died before they could be subjected to further tests.

In the last case, the only marker of the irradiated X present was $car^+$, near the base of the left arm, hence the distal portions, at least, of both arms had been removed. The obvious way for a curtailed chromosome of this type to survive is by ring formation. It is alternatively conceivable, however, by postulating a third break, this time in an autosome; this would imply further that the surviving $car^+$-bearing segment did not include the centromere of the X, and that it became inserted into the autosome where the latter had broken, while the centromere-bearing fragment of the X became lost by the breakage-fusion-bridge cycle.

The experiments with both the Bar-M1 and scute-V1 stocks have given such a paucity of cases (5 in a total of 23) which could plausibly be interpreted as having resulted from single healed breakages that, in view of the other readily available interpretations of these cases, it does not seem justified to consider the experiments as indicating the occurrence of such a class of mutations. On the contrary, the data can be used to argue against the frequent healing of X-ray induced breakages. For, if healing of breakages is not a rare phenomenon (and it would not be according to Fano's reckoning of 1941 that as many as 5 per cent of single breakages heal), then in view of the additional breakage and union required for two-break deletions and the lower viability of many of the individuals carrying them, caused by the additional hyperploidy due to the genes located in the added left-hand region, as compared with the lesser hyperploidy of individuals with single healed breakages, one might expect that the number of cases of apparent healing would equal if not exceed the number of cases identifiable as two-break deletions.

## INVESTIGATION OF THE HEALING OF BREAKS INDUCED IN ATTACHED-X CHROMOSOMES IN OŎCYTES

For our experiments on the breakage of attached-X chromosomes in females, a special type of attached-X chromosomes was constructed, having the markers *sc* $ct^n$ *oc car* in one arm and *y In49* $sn^{x2}$ in the other, and carrying no Y chromosome (see fig. 1). This stock is known by the abbreviation "snoc," derived from the recessive female-sterilizing genes, $sn^{x2}$ and *oc*, located in its opposite arms. The males of this stock (not used in the experimental crosses) are of the type having both parts of the Y attached to the X (abbreviated as "X·Y") constructed by Lindsley and Novitski (1950), so that no free Y need be present in either sex. After the present experiments were completed, females from cultures of this stock were tested to determine whether they had somehow obtained a Y, but the tests showed no Y to be present in them.

This stock maintains itself with high stability except for the very rare appearance of homozygous *y* or *sc* flies, derived from crossing over distal to *In49*, which are readily recognized and discarded, and for the more frequent appearance of homozygous *y* $sn^{x2}$ or *sc* $ct^{n}$ *oc* (with or without *car*), derived from crossing over proximal to *In49*. The latter types are, however, self-eliminating because of the sterility of homozygous $sn^{x2}$ or *oc*, as the case may be, these genes having been introduced mainly because of this stabilizing effect on the stock. Besides these types of crossovers, there are some caused by *car* changing places between the arms of the X's or becoming replaced by homozygous *car* (easily distinguishable) or $car^{+}$, but these changes would cause no serious complication in experiments of the present type. The reason for having the ends of the two arms differentiated by *y* and *sc* respectively is to provide them with markers whereby the presence of either, both, or neither chromosome end may be distinguished, according to whether the phenotype of an exception is $y^{+}$ sc, y $sc^{+}$, $y^{+}$ $sc^{+}$, or y sc, respectively.

Virgin females of the "snoc" stock were irradiated with approximately 2000 r and then crossed with $B/Y^{+}$ males. The parents were transferred to fresh bottles periodically (according to a scheme shown in table 3), and the offspring examined for exceptional males and females. The unexceptional flies were *B* sons (sterile because no Y chromosome was contributed by the mother), wild-type daughters (like "snoc" females but possessing a Y chromosome), or cross-over daughters (of various phenotypes, none of which however show barring of eyes).

The main types of exceptional male individuals caused by breakage and detachment between the major parts of the attached X's would have the following phenotypes:

1. $ct^{n}$ oc car and $sn^{x2}$ males. These represent long paracentric interstitial deletions where the subterminal break occurred proximally to $sc^{+}$ and $y^{+}$, respectively (see fig. 2A).

TABLE 3

THE DISTRIBUTION OF EXCEPTIONAL AND UNEXCEPTIONAL $F_1$ FLIES IN DIFFERENT BROODS OBTAINED AFTER TREATING "SNOC" FEMALES WITH ABOUT 2000 r OF X-RAYS AND CROSSING THEM TO $B/Y^{+}$ MALES

| Brood No. | 1 | 2 | 3 | 4 | 5 | 6 | 7 | Totals |
|---|---|---|---|---|---|---|---|---|
| Brood length (in days) | 2 | 2 | 3 | 4.5 | 3.5 | 3 | 9 | |
| No. of bottles | 15 | 15 | 15 | 15 | 9 | 9 | 9 | |
| No. sc $ct^{n}$ oc(car) ♂♂ | 2 | 6 | 3 | 1 | 1 | 1 | 0 | 14 |
| No. y $sn^{x2}$ ♂♂ | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| No. "B" ♀♀ | 25 | 44 | 31 | 11 | 9 | 6 | 3 | 129 |
| No. unexceptional ♂♂ (B) | 1347 | 2745 | 3553 | 3909 | 2495 | 2141 | 1801 | 17,991 |
| No. unexceptional ♀♀ ($B^{+}$) | 1059 | 2343 | 3072 | 3296 | 1995 | 1908 | 1423 | 15,096 |
| % crossovers among $B^{+}$ ♀♀ | 7.2 | 5.7 | 5.5 | 7.3 | 10.0 | 9.9 | .... | |

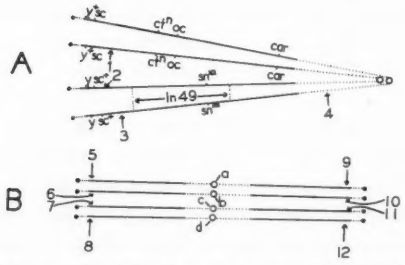


FIGURE 2. A. Scheme of origination of paracentric deficiencies during tetrad stage, illustrated by cases in which one break is located proximally (4) and the distal break may occur in any one of three positions (1,2,3). B. Diagram of an autosome in the tetrad stage, with possible subterminal points of breakage indicated by numbers 5–12.

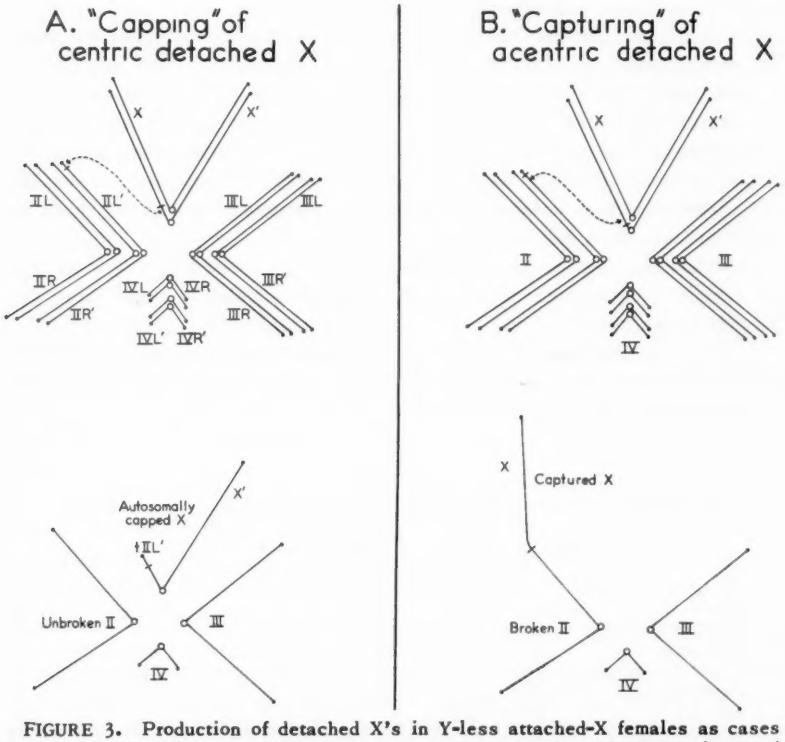


FIGURE 3. Production of detached X's in Y-less attached-X females as cases of "half translocations." Chromosome groups above are that of oocytes in tetrad stage; the groups below are those of haploid female pronucleus of ovum, (positions of breaks indicated by cross lines, of attachments by dotted lines with arrows). Both attachments may occur at once, reciprocally, in the oocyte, but for the production of phenotypically recognizable detached X only one or the other "half" of the reciprocal translocation may enter the egg.

2. sc $ct^n$ oc car and y $sn^{x2}$ males. These result, conceivably, from one of the following 5 major causes: (a) Single healed breakages in or near the proximal heterochromatin of the arm opposite that represented. (b) Paracentric interstitial deletions in which the subterminal break occurred distally to $sc^+$ or $y^+$. (c) Ring chromosomes formed by union of a proximal break with one in the terminal heterochromatin of the opposite arm, or (d) and (e) translocations of the two main types discussed on pp. 200 to 203 and shown in figs. 2 and 3. The exceptional females resulting from detachment would all be recognizable by their barred eyes, which would be of heterozygous type. They could have any of the origins already mentioned for the exceptional males. In addition, homozygous barred eyed females are produced by sperm carrying non-disjoined X's fertilizing eggs containing no sex chromosome.

The types of $F_1$ flies obtained are given in table 3. There were 129 bar-eyed females in 15,096 $F_1$ females examined and 14 sc $ct^n$ oc car and 1 y $sn^{x2}$ males among the 17,991 $F_1$ males examined. The results thus far are in complete confirmation of those obtained by Rapoport (1940). For while no exceptional male identifiable as resulting from an interstitial deletion was obtained, 15 cases were found of males showing apparent healing of breakages. The analysis of the females, however, was continued a step further than was Rapoport's (1940). An attempt was made to breed all bar-eyed exceptional females (which happened to be virgin, since all males but the 15 exceptional ones were sterile). Since these females differed in the degree of bar eyes manifested and some had cut or notched wings, their phenotypes and the number of them tested (a total of 61 of the 129) are shown in table 4. Of the 13 narrow-bar-eyed females tested, 10 produced sons all of which were phenotypically bar, proving these females were the result of non-disjunction of the X chromosome in their fathers. (It is very unlikely, because of their scattered distribution, that they were the result of the accidental presence of a Bar female among the Bar parental males used.) If none or all of the other 6 untested phenotypically similar females were of the same origin, the frequency of this type of non-disjunction would range from 5.6 to 8.9 $\times 10^{-4}$ (10-16/17,991), a range in

TABLE 4

PHENOTYPIC CLASSIFICATION AND FATE OF EXCEPTIONAL "B" ♀♀ IN TABLE 3

| Phenotype | No. fertile | No. untested (No. dead and infertile + No. fertile but untested) | No. tested* | Total No. |
|---|---|---|---|---|
| "Homozygous Bar" "Weak Bar" and/or "cut" wings | 13 | 6 + 0 | 13** | 19 |
| "Heterozygous Bar" | 6 | 22 + 5 | 1 | 33 |
| Total | 50 | 24 + 3 | 47 | 77 |
| | 69 | 52 + 8 | 61 | 129 |

*See text for methods and results of testing.

**Ten of these proved to be B/B ♀♀ due to paternal non-disjunction.

frequency similar to that found in other stocks. The great majority of the group of 33 females with very slightly barred eyes and/or cut or notched wings were practically sterile and poorly viable, probably because they were too heteroploid. Thus it could not be determined whether most of them were ordinary triplo-X's or resulted from single or multiple breakages. However, control crosses using unirradiated females, which would show triplo-X's as frequently, were not made.

If females containing a paternal X chromosome plus an X derived from breakage of the maternal "snoc" attached-X's are mated with *y sc* males the offspring furnish information as to the source and origin of the maternal X. If non-crossover sons bearing the "snoc"-derived X chromosome survive, the analysis of the breakages which produced them is the same as that given before for the cases of $F_1$ exceptional males. Even if such sons do not survive an analysis can be made from a study of the other offspring. A *sc* $ct^n$ *oc car* chromosome, even if it contains a lethal, will when in association with the *B*-containing chromosome in the exceptional female give crossovers, resulting in sons having the above markers in smaller groups, some of the daughters also will show sc and they will show that crossing over has occurred freely between *sc* and *B*. If on the other hand the exceptional Bar female contains a *y In49* $sn^{x2}$ chromosome, some of the daughters will be y, but there will be very few crossovers between *y* and *B* (because of the *In49*); the sons will show y sn only if there is no lethal in that region. In case the exceptions have arisen by deletion, and the terminal marker is thereby "covered," it becomes uncovered by crossing over between it and *B*, or between *B* and the centromere. Even when *In49* is present, enough crossing over of this kind occurs for the purpose.

A summary of the results of the analysis of 51 such exceptional $F_1$ females is given in table 5. Six cases were found of a two-break deletion, in which one break was to the right of $sc^+$ or $y^+$ and the other in the proximal region of the same arm, so that all the markers of one arm showed phenotypically except the left-most, which was "covered" by the deleted piece. It becomes clear that the failure to obtain any exceptional males with deletions of this type in the $F_1$ in these experiments is due in considerable measure to the rarity of viable exceptions of this type in comparison with the viable breakage exceptions of the other type, the numbers of

TABLE 5

RESULTS OF ANALYSIS OF 51 EXCEPTIONAL B ♀♀ RESULTING FROM BREAKAGE OF THE "SNOC" CHROMOSOME

| Chromosome derived from "snoc" | Apparent "healing" | | Multi-break deficiency | |
|---|---|---|---|---|
| "Phenotype" | sc $ct^n$ oc(car) | y $sn^{x2}$(car) | $ct^n$ oc car | $sn^{x2}$ |
| No. viable in sons | 9 | 22 | 1 | 1 |
| No. inviable in sons | 5 | 9 | 2 | 2 |
| Total | 45 | | 6 | |

the two types among exceptional females carrying chromosomes resulting from breakage and viable in the sons being 31 and 2, respectively, a ratio of 15½:1. Since among the $F_1$ exceptional males the corresponding numbers were 15 and 0 it is evident that there is no discrepancy here, the difference between these observed ratios being entirely without significance. In later work, being reported separately by Herskowitz (1954), a higher proportion of deletion cases than 1 to 15 was found among the $F_1$ males. Since, however, there are degrees of inviability of the deletional exceptions, depending on the size of the extra piece, and since any extra piece derived from an X is known to be more damaging to the male than to the female carrying it, because of the greater ratio unbalance of genes thereby caused in the male, it will follow that more of the female than of the male deletional exceptions will survive, relatively to the nondeletional exceptions, i.e. the observed ratio of non-deletion to deletion exceptions is actually expected to be lower among the exceptional $F_1$ females than males.

Even allowing for this, however, and especially in view of the later results of Herskowitz above referred to, it is difficult to understand why in Rapoport's experiments no deletional exceptions whatever were found among the reported 69 $F_1$ males resulting from breakage. Possibly culture conditions caused very adverse selection against the weaker deletion-type individuals, and the *Hw* in these individuals may have played a considerable role in weakening them; alternatively, detection of the *Hw* (which however was expected to be accompanied by $y^2$ in place of *y*) might have been difficult, especially in the presence of *fa* and *f*.

The influence of the extra piece derived from the left end of the deleted X in depressing the viability of the exceptions carrying it is shown by the fact (see table 5) that 4 of the 6 exceptional females resulting from obvious deletion gave results showing the actual lethality of their exceptional X chromosome in the male, whereas only 14 of the 45 exceptional females which appeared to result from some other type of structural change proved to be fully lethal in the male. The question still remains, however, why even as many as 14 out of 45 (approximately a third) of the chromosomes of the latter type were lethal, when the dose of 2000r, even if given to spermatozoa, would have produced only about 5½ per cent of lethals (approximately an eighteenth), and the frequency when the female is treated is lower. The answer obviously is because in the great majority (if not in all) of these 45 cases the break in the proximal region of the arm which was lost was not exactly adjacent to the centromere, but left some of the proximal region as a duplication. There are results indicating that even the heterochromatin of the X is somewhat damaging to life when present in excess, but in some of the cases the breaks would have been in euchromatin, and when these euchromatic pieces are long enough they become lethal to the male while still only "detrimental" to the female, according to the principle previously referred to, of the greater genic imbalance caused by additions of pieces of the X to the male than to the female. Thus the lethality here is fundamentally of the same nature as that occur-

ring in the case of obviously deleted X chromosomes, but the latter are still more subject to it, since in their case both the left and the right subterminal regions of the X can participate in producing it.

The fact that, as table 5 shows, only 15 male exceptions were found, but that 33 of the analyzed female breakage exceptions, and doubtless others which were not analyzed, carried chromosomes which were viable in the male, is only in line with expectation. For the male exceptions were subject to much more adverse selection than the female exceptions, both because of the more damaging effects of X-chromatin hyperploidy on males, already referred to, and also because of the detrimental effects of their recessive markers, since these were phenotypically manifest. The latter damage was particularly great in the case of the very weak y $sn^{x2}$ flies, and explains why male exceptions of this class ran so far behind those of the contrary class.

On the other hand, there is no obvious reason for the great and significant excess of analyzed female exceptions carrying *y* $sn^{x2}$ chromosomes over those carrying *sc* $ct^{n}$ *oc car* chromosomes (the numbers being 31 and 14, respectively). As it is unlikely that their markers in only heterozygous condition, would have this effect (especially since the *y* $sn^{x2}$ is so much weaker when hemizygous), the results suggest that perhaps the heterochromatin of the arm in which *y* $sn^{x2}$ lies may be longer or more breakable, or, if broken, more likely to undergo union of the type appropriate to give these exceptions, than the heterochromatin of the other arm. In the light of the mechanism by which most attached-X's have arisen (by "crossing over" with the Y, such "crossing over" between heterochromatic regions being notably inaccurate), and of some cytological observations of attached X's, it is reasonable to regard their heterochromatic regions as being in many cases not exactly matched, and therefore likely to have somewhat different tendencies to undergo structural changes.

Discounting the ten proven cases of paternal non-disjunction, and the 27 "weak Bar" and/or "cut-like wing" sterile females which were probably triplo-X or nearly triplo-X "superfemales" there were 92 exceptional females of which 6, or 5 per cent, could be shown to involve multiple breakages of the X. Five of these 6 came from the first two broods of eggs deposited. Although at first glance the number of such cases seems so small as to invalidate the interpretation that the rest of the 92 might also be due to interchange types of structural change, this conclusion would be premature. It must in the first place be recognized that the 6 exceptional females which were proven by the use of the genetic markers to have resulted from double-break X-chromosome deletions, and not from the healing of single breakages, must represent a minimum number which we may call class (1), in relation to all cases of "detachment" caused by two breaks both lying somewhere in the X's. To this minimum should be added an undetermined number of simulated healings which were really (2) paracentric interstitial deletions of orthodox type in which however the distal break was distal to the markers $y^{+}$ or $sc^{+}$; (3) pericentric deletions in which

one break occurred in the proximal region of one arm and the other in the distal heterochromatin of the other arm (distal to any genes necessary for life or normal appearance), forming a ring X-chromosome somewhat like those denoted as $X^{c1}$ and $X^{c2}$; (4) deletions which we may term "homotelic," in which one break occurred in the proximal region of one arm (in position 4, in fig. 2A), and there became attached to the "stump" of this arm, at its breakage point, a subterminal region broken off of the sister chromatid of the unbroken arm (at position 1 in fig. 2A). In this case the two subterminal regions are genically identical.

In further elucidation of class (4) it is to be observed that if the broken arm had *y* in its subterminal region, the broken arm would be "capped" by a subterminal region which also contained *y* (except in the comparatively rare case of the break having been distal to *y*), and the phenotype would remain y (this being true also if the break was distal to *y*). Similarly, if the *sc*-containing arm were the unbroken one, the phenotype would remain sc (although not so extreme sc because of the cumulative action of the added hypomorphic gene). Hence these cases also would simulate simple breakage with healing. Certainly sister chromatids, participating in tetrads, are present during most of the oocyte period, when the great majority of the breakages for our exceptions were produced. If it is largely a matter of chance which of the three possible members of the X tetrad contributes the subterminal piece (the fourth member, consisting of the unbroken arm which survived, being of course impossible as the contributor), then approximately one in every three of the paracentric deletions was of this nature. For the tip could be derived either from the sister chromatid to the arm which survived unbroken (by a break in position 1 of fig. 2A), so as to give the homotelic deletion, or from the distal portion of the broken arm whose proximal stump survived (the break being in position 2) or from the sister chromatid to the one which provided the stump (the break being in position 3).

Thus to the six cases of demonstrated deletions (our type 1) there should be added about three cases of type (4) (homotelic), and an undetermined—but undoubtedly smaller number of types (2) and (3). Moreover, since the six cases were of course all derived from the 47 *tested* cases, while in fact there was a total of 77 (or conceivably as many as 92) observed cases (tested plus untested) of heterozygous Bar exceptions, or at least $\frac{5}{3}$ as many in all as the tested cases, it turns out that there were probably at least $\frac{5}{3} \times 9$, or 15 deletional exceptions among the approximately 15,000 $F_1$ females counted, namely, 1 in 1000. Considering that the dose was only 2000r, and that there were doubtless many more deletional exceptions whose hyperploidy prevented their survival than those which were recovered, the rate of their production appears unexpectedly high, in relation to the frequency of production of gross rearrangements which has ordinarily been attributed to females.

However, 15 still falls far short of the total number of exceptional females with a detached X, whether we take that total to be 77 or some larger number (even though if we took the total as larger the number of deletional

exceptions would also be reckoned as proportionally larger). It remains to consider what the origin probably was of these other exceptions, all of which phenotypically resemble the products of single breakage followed by healing.

As soon as it is recognized that the chromosome "tip," i.e. the broken off subterminal (including terminal) region, which in a case of deletion of an attached X becomes joined to the "stump" of the arm that was broken proximally, may have been derived from any one of three of the four chromatids of the X tetrad, it becomes evident that the source of the tip could just as well have been an autosome. Since there are three pairs of autosomes (i.e. 6), each with two ends, and since, in the oocytes in which those breakages usually occur which give us our exceptions, the chromosomes are already divided into chromatids, there are 24 autosomal chromatid tips to afford sources for the "capping," if we may so term it, of the stumps of the broken X's. (See fig. 2B, showing the tips available in one tetrad.)

However, in the case of any given type of tip, such as for example the left end of chromosome II, whichever chromatid furnishes the tip which "caps" the stump of the X must not itself be the chromatid (at least, not in the region where the break occurred) to enter the egg, along with the "capped" detached X, if the result is to be a phenotypically recognizable, viable, exceptional individual, simulating the result of a healed single break. For, if that autosomal chromatid at its subterminal breakage point had united with the acentric detached X or piece of X, by reciprocal translocation, and this combination had been present in the egg along with the other parts of the same broken X and autosomal chromatids, there would have been nothing phenotypically to distinguish the viable resulting offspring (that formed when the sperm carried no X) from the mass of unexceptional females. And if, alternatively, the autosomal chromatid had failed to meet and join with the acentric detached X and then entered the egg with the capped centric detached X, the resulting breakage-fusion-bridge cycle might be expected to cause the death of the zygote, either by bridge formation or, if not in that way, then by the hypoploidy occasioned by the absence of almost an entire autosome. A partial exception must be made here in the case of chromosome IV, since haplo-IV's are capable of surviving, yet they are late in developing, of Minute bristle phenotype, and comparatively low in viability. By contrast with the situation for the chromatid which furnishes the tip to cap the stump of the centric detached X, any of the other three chromatids, if entering the egg along with that detached X, will result in an exceptional individual, as shown in fig. 3A. This exception will be of the type simulating a healed single break, since of course the recessive marker carried by the distal tip of the unbroken arm of the X will not be "covered" by the autosomal tip that is attached to the stump.

Since the tip provides a third "dose" of its contained genes, which are already represented in the two unbroken autosomes of the given type possessed by the exceptional individual, there are size limitations on the tip

that can take part in the production of a *viable* exception. These limitations are dependent upon the degree of damage to life caused by hyperploidy of the distal region in question, as its length is increased in a series of cases having breaks successively less distally placed. Thus given equal chances of breakage and recombination for equal lengths of different subterminal regions, the relative frequencies of observed exceptions involving them would be proportional to the values obtained for each of them when a summation was made of the viability of hyperploids resulting from each possible position of breakage, from just proximal to the telomere to the most proximal position that yielded any viable exceptions. Present knowledge of these facts is extremely fragmentary, but it is known that hyperploidy of the entire fourth chromosome does not depress viability greatly, and that hyperploids for "tips" of the major autosomes so frequently survive, when derived by recombination from individuals having translocations between these autosomes and the Y, as to suggest that hyperploids for fairly large subterminal regions retain a moderate viability. The autosomal subterminal hyperploids, unlike those of the X discussed previously, should show no consistent tendency to be more viable in one sex than the other.

We have seen that, just as for the production of a deletional exception a tip must be derived from one of three chromatids of the X and not from the fourth, so for the production of a translocational exception of the above "autosomally capped X" type, where the centric part of the detached X receives an autosomal tip, only three of the four tips of any given type (excluding that of the chromatid whose strand proximal to the break enters the egg) may take part. In other words, in effect, there are only ¾ of 24, or 18, available autosomal tips for these exceptions. If then autosomal tips were (1) as likely to break off and become attached to the stump of the X as are tips of the X themselves, and (2) had a similar distribution of viabilities per length, and (3) if, further, there were no preferential segregation of chromatids with respect to attached tips on X's there should be 18 translocational exceptions of the autosomally capped X type just described to 3 deletional exceptions, or 6:1. But since, of the 3 deletional exceptions, only 2 (and not always these!) reveal their deletional nature by their distal marker combination ($y^+$ $sc^+$), there would result 19 exceptions simulating single breakage with healing to 2 phenotypically recognizable deletions, or 9½:1. This is an even higher ratio (though not significantly so) than that of 45:6 (or 7½:1) which was found on analysis of 51 female exceptions (table 5). Doubtless the similarity of these observed and "expected" ratios is largely accidental, however, since there is no good reason to suppose that all 3 of the above mentioned conditions, required for the calculation of this expectation, are strictly in conformity with the actual situation.

It should however be mentioned that the third condition, that of an approximately random segregation of the autosomal chromatid region proximal to the break, with respect to the segregation of the tip-$X^-$ combination,

probably holds nearly true, at least for the major autosomes. For these tips are too small and too far removed from the centromeres of the major autosomes to be able to affect the direction of segregation of these centromeric regions, and so much crossing over must have occurred between the centromere and the break to have randomized the distribution of the region just proximal to the break, with respect to the distribution of the centromeric region of the same chromatid. If however the autosomal arm has at the same time acquired by reciprocal translocation the acentric piece of the X (a contingency which may be rare in oocytes), that might conceivably exert an influence on the segregations of the pieces in question. But it does not seem likely that the frequency of observed exceptions of these kinds could be influenced very radically by such means.

It remains to be pointed out that, theoretically, there is in our material still another category of detachments which, although simulating healed breaks, arise by double breakage followed by union of broken ends: namely, that which we may entitle "captured X's." These involve the attachment, at its breakage point, of an acentric broken X to the breakage point of the centromere-bearing portion of an autosome, from which a small subterminal piece has been broken off (see fig. 3B). The X then dangles, as it were, on to what appears to be the end of the autosome. This is the reciprocal of the type of "capped X" cases which we have been considering. Moreover, when reciprocal translocations occur, both these complementary events (capping and capturing) can take place in the same cell. However, it is only when an egg receives but "one half" of such a translocation, or when only one of the two reciprocal unions has occurred and the egg has received the products of it, that a phenotypically recognizable exception is produced. Theoretically, the cases of captured X's probably occur as often, if we confine our attention to their initial stages, present in the oocytes, as those of autosomally capped X's.

What proportion of the oocytes containing captured X's results in viable eggs giving recognizable exceptions is a matter that cannot be decided without experimental evidence of the influence which captured X's have on the segregation of free X's, of both detached attached-X types. It would take us too far afield to discuss the possibilities here, but there is some ground for thinking that a captured X would exert some influence in "repelling" (i.e. segregating to the opposite pole from) the centric X from which it had been detached, and also in repelling the still attached X of the original tetrad structure. If so, an egg receiving a captured X would have a better than $\frac{3}{4}$ chance of acquiring the chromosome combination necessary for the production of a recognizable exception, and in this respect captured X's would be more likely than capped ones to give recognizable exceptions.

However, the number of observable cases of captured X's must be far smaller than that of autosomally capped X's, because (with the possible exception of some cases involving chromosome IV, where non-disjunction

might lead to viable hyperploidy of a part of that chromosome), the exceptions with captured X's are hypoploid (haploid) with regard to the subterminal piece of the autosome (see fig. 3), whereas the exceptions with autosomally capped X's are hyperploid for the subterminal piece. Since, in general, hypoploidy of a given length of chromatin involves more ratio change in genes than hyperploidy does, and is known, on the basis of plentiful evidence, to be much more damaging to life, it is evident that recovered exceptions (i.e. those that have survived) of the captured X type will be much more limited in the size of the aneuploid subterminal piece of the autosome (i.e. in the distance from terminus to break) than will exceptions of the capped X type. Therefore the frequency of observed cases of captured X's will be much smaller than that of autosomally capped X's. (Again a partial exception must be made of cases involving chromosome IV, since the entire left arm of this, being heterochromatic, has little effect on viability no matter whether haploid or triploid, and even the entire right arm is somewhat viable, though of Minute phenotype, and when haploid.) Nevertheless, the captured-X cases will necessarily swell to some extent the total frequency of observed cases of detachment that simulate healed breakage, as opposed to the cases of obvious two-break deletions.

On the basis of the various considerations presented above, and in the absence of specific experimentally obtained knowledge of the breakage and attachment proclivities of the subterminal regions of the different chromosome arms, and of the relative viabilities of aneuploids involving them, one would estimate that the ratio of detached-X cases simulating healed single breaks to those of obvious double-break deletions would be appreciably higher than 9½:1. The observed numbers of 45 to 6, or 7½:1, among such cases, although not to be regarded as a confirmation of the equal participation of all chromosome arms in the production of the detachments, do show a high enough proportion of deletions to make quite unnecessary the interpretation that any of the cases were actually results of single breaks which underwent healing, and thus remove the supposed experimental basis for that view derived from experiments with attached X's.

Supposing that all the exceptional individuals did result from double or multiple breakage, with fusion at the points of breakage, gross rearrangements of this type (not counting the ones whose aneuploidy was great enough to prevent their survival) occurred at a frequency of about 2 per cent in the first two broods of offspring examined (table 3)—a far higher frequency than is usually thought to be produced in females by only 2000 r. In the third brood (4th to 7th day), representing mainly earlier oocytes, the frequency was somewhat lower, and in the still later broods, representing cells that were oogonia when irradiated, the frequency fell much lower still. The recognizable deletions, taken by themselves, show the same trend. These results bring gross rearrangements into line with the finding by Muller, R. M. Valencia and J. I. Valencia (1949), based on data on mutations involving specific loci, that deletions are produced with a higher

frequency in late oocytes than in early oocytes or oogonia, although in their work there was no evidence of gross rearrangements (involving the specific loci) having been produced.

## CONCLUSIONS CONCERNING HEALING OF CHROMOSOME ENDS AFTER BREAKAGE

Consideration of the previous evidence and of the data obtained in the present studies, shows that there is no cogent evidence in favor of the occurrence of mutations which change the polarity either of interstitial or telomere genes in Drosophila males or females. This does not mean that such changes can never be produced or detected by other methods, and less so, that such mutations have never occurred in the evolution of this species. The evidences for such polarity changes in other species have been summarized elsewhere (Muller, 1954), and it would require too lengthy a discussion to reconsider them here in a satisfactory manner. In particular, the healing of broken chromosome ends appears to be a regular, perhaps adaptive, phenomenon in sporophytic tissues of maize, as shown by the work of Stadler, McClintock and others. The present findings, however, do reaffirm the position (Muller, 1938, 1941) that in Drosophila the polarities of genes and chromosomes have become stabilized to such a degree as to justify the naming of the monopolar ends of chromosomes as telomeres, and their terminal genes as telomere genes. Moreover, the similarity of the gross chromosome changes induced by radiation in mice, grasshoppers and the other animals studied to those observed in Drosophila suggest that the same principle may extend to them.

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## SUMMARY

The numerous earlier cases in Drosophila which had been thought to illustrate changes in gene polarity—i.e. the conversion of interstitial genes to telomeres ("healing") and *vice versa*, and occasionally, the formation of branched connections—have on more searching analysis given evidence of involving, instead, only the union of the broken ends of chromosomes in a new arrangement. Moreover, several large-scale earlier experiments, designed to test the occurrence of healing by means of quantitative studies of the effects of irradiation of spermatozoa, have failed to give evidence of healing. Such studies have included attempts to obtain healing both in eu- and in hetero-chromatin, when the broken ends had resulted from X-rays, from ultraviolet, or from the mechanical rupture of dicentric bridges. It is true that, since the resolution of most of the earlier moot cases, some additional cytological evidence suggesting the conversion of interstitial genes to telomeres and *vice versa* in Drosophila has

been reported, but these later cases have not been reinvestigated in the light of modern knowledge of the possibilities, and they must be regarded as far from conclusive.

New attempts are herein reported to obtain evidence regarding the healing of breakages in chromosomes derived from spermatozoa exposed to some 4000 r of X-rays. Two types of X-chromosomes, especially suitable for the detection of such changes, were used, namely Bar-M1 and the synthetic (crossover) chromosome designated as y·Dp($sc^{V1}$), explained in the text. Only 5 cases which might have represented healed breaks, as compared with 18 identified multi-break cases, involving changes in the connections of broken ends, were obtained. This frequency for the cases of possible healing is too low to afford evidence in favor of this phenomenon, in view of the fact that in our experiment a number of types of interchange of connections of broken ends, which collectively could well have been as frequent as this, would have produced individuals of the given phenotypes, identical with those which would have been produced by healed breakage.

Pursuant of the articles by Rapoport (1940, 1941, but only recently available to us), reporting the formation of detached X-chromosomes as a result of the healing of breakages when Drosophila females with attached-X's and no Y were X-rayed, a similar study has been carried out by us. Special attached-X's were constructed, of a type designated as "snoc" (see text), which were particularly suitable for the finding of such cases. Females with these X's and no Y were exposed to some 2000r of X-rays. The data obtained in the $F_1$ completely confirm Rapoport's empirical findings.

However, genetic analysis of 51 of the exceptional $F_1$ females, containing detached X's, proved that in at least 6 cases these X's had resulted from paracentric interstitial deletions. It is shown that this number of deletions is certainly a minimum one, and that the frequency of the intra-X interchanges in this material is relatively high, in view of the dearth of cases of all types of interchanges hitherto reported as having been obtained from females. Even more surprising is the consistency of the data with the interpretation that all the exceptional cases, in both Rapoport's and the present study, resulted from interchanges of connections of broken ends, rather than healing. For the consideration must not be neglected that when (as was the case for most of the exceptions here produced) oocytes are irradiated, the broken proximal portion of the X is not restricted to becoming joined with a subterminal portion broken off from the chromatid of an X chromosome, so as to give a deletion type exception, but that it may become joined, instead, with a subterminal region broken off of any autosomal chromatid. This would result in a translocational-type exception phenotypically like that expected from healing.

Accordingly, the results from X-rayed females can no longer be considered as evidence for the healing of breakages with formation of new telomeres. At the same time, on the interpretation that the detachments obtained do represent interchanges of broken ends, it must be concluded that the frequency of this phenomenon in oocytes is much higher than had been

suspected. Moreover, the data indicate that irradiated oocytes yield many more of these gross rearrangements than do irradiated oogonia.

It is concluded that, under the wide variety of conditions in which healing of breakages has been sought in Drosophila it either does not occur or else occurs with viable products so rarely as to escape detection by quantitative means.

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